



Title	Molecular mechanisms of liver regeneration and protection for treatment of liver dysfunction and diseases
Author(s)	Fujiyoshi, Masato; Ozaki, Michitaka
Citation	Journal of Hepato-Biliary-Pancreatic Sciences, 18(1), 13-22 <a href="https://doi.org/10.1007/s00534-010-0304-2">https://doi.org/10.1007/s00534-010-0304-2</a>
Issue Date	2011-01
Doc URL	<a href="http://hdl.handle.net/2115/44870">http://hdl.handle.net/2115/44870</a>
Rights	The original publication is available at <a href="http://www.springerlink.com">www.springerlink.com</a>
Type	article
File Information	JHBPS18-1_13-22.pdf



[Instructions for use](#)

*Molecular mechanisms of liver regeneration and protection*

*- for treatment of liver dysfunction/diseases -*

Masato Fujiyoshi and Michitaka Ozaki

Department of Molecular Surgery,  
Hokkaido University School of Medicine,  
N-15, W-7, Kita-ku, Sapporo, Hokkaido, 060-8638 Japan

*Key words:* liver regeneration, liver protection, apoptosis, IL-6/STAT3,  
PI3-K/PDK1/Akt

Received on December 31, 2009.

*Correspondence to:*

Michitaka Ozaki M.D., Ph.D.  
Department of Molecular Surgery,  
Hokkaido University School of Medicine,  
N-15, W-7, Kita-ku, Sapporo, Hokkaido, 060-8638 Japan  
E-mail address: [ozaki-m@med.hokudai.ac.jp](mailto:ozaki-m@med.hokudai.ac.jp)  
Telephone: +81 11 706 7063  
Fax: +81 11 717 7515

## ***Abstract***

Liver regeneration is a necessary process which most liver damage depends on for its recovery. It is achieved by a complex interaction network consisting of liver cells (hepatocytes, Kupffer cells, sinusoidal endothelial cells, hepatic stellate cells and stem cells) and extra-hepatic organs (thyroid gland, adrenal gland, pancreas, duodenum and autonomous nervous system). The restoration of liver volume depends on the hepatocyte proliferation which includes initiation/proliferation/termination phases.

Hepatocytes are 'primed' mainly by Kupffer cells via cytokines (IL-6 and TNF-alpha) and then 'proliferation' and 'cell growth' of hepatocyte are induced by the stimulations of cytokines and growth factors (HGF and TGF-alpha). Liver regeneration is achieved by cell proliferation and cell growth, where IL-6/STAT3 pathway and PI3-K/PDK1/Akt pathway play pivotal roles, respectively. IL-6/STAT3 pathway regulates hepatocyte proliferation via cyclin D1/p21 and protects against cell death by up-regulating FLIP, Bcl-2, Bcl-xL, Ref1 and MnSOD. PI3-K/PDK1/Akt is known to be responsible for regulation of cell size via its downstream molecules such as mTOR in addition to its survival, anti-apoptotic and anti-oxidative properties.

Although the molecular mechanisms of liver regeneration have been actively studied, it is required to elucidate and leverage the mechanisms of liver regeneration for the

sufficient treatment of liver diseases.

## *Introduction*

Even though we are in the advanced era in hepato-biliary-pancreatic surgery, the recovery from most liver damage depends mainly on the innate regenerative capacity of liver. The regenerative processes of liver are complicated. It can be induced/triggered not only by the primary reduction of liver mass such as partial hepatectomy (PH) or liver transplantation with small-for-size grafts, but by the secondary reduction of viable liver mass induced by injurious liver diseases such as viral hepatitis, drug injury or steatosis[1]. In such pathological situations, liver regeneration often progresses in parallel to gradual damage of liver parenchyma and structure. Therefore, for the sufficient treatment of liver diseases with the maximal liver regeneration/function, it is required to elucidate and leverage the biological mechanisms of protection as well as regeneration of the liver under normal and pathological situations.

In this review, we describe the molecular mechanisms of liver regeneration and protection in rodents and discuss the functions of some critical molecules (pathways) of hepatocytes. Especially, we focus on the distinct roles of IL-6/Jak/STAT3 pathway and PI3-K/PDK1/Akt pathway on cell proliferation, growth (increase of cell size) and protection (anti-oxidant and -apoptosis) during liver regeneration.

## *Molecular mechanisms of liver regeneration*

### *General concepts on extra-/intra-hepatic regulation of liver regeneration*

Liver is composed of a various types of cells, hepatocytes, Kupffer cells, sinusoidal endothelial cells (SECs), hepatic stellate cells (HSCs) and stem cells[2], which develop complex network with intimate interactions inside the liver[3]. And the well organized intra-hepatic network is functionally connected with the extra-hepatic organs throughout the body such as thyroid gland[4-5], adrenal gland[6-7], pancreas[8], duodenum[9] and autonomic nervous system[10]. The extra-hepatic organs and systems synchronously and cooperatively regulate the functions/behaviors of liver and liver cells through some hormones (insulin, glucagon, T3, norepinephrine and somatostatin) and growth factors (EGF).

The intercellular interactions between liver cells are mediated by growth factors (HGF, TGF-alpha and beta) and cytokines (IL-6, TNF-alpha and IFN-gamma ) [2-3, 11]. This inter-dependent control system seemingly achieves the precise and robust regulation of liver regeneration. These growth factors and cytokines will immediately and synchronously evoke various cellular events essential for liver regeneration/protection by sending signals finally to hepatocytes. This network system of liver regeneration is possibly comprehended as the spatio-temporal dynamic changes

of the signal transduction systems in the liver. The temporal development of the specifically activated signaling pathways during liver regeneration may be divided into three phases, initiation phase, proliferation phase and termination phase[2]. In each phase, some characteristic cellular/molecular events have been observed. However, the precise signaling pathways in hepatocytes and their functional roles during initiation, proliferation and termination of liver regeneration have not been understood well. We will focus on the roles of signals, especially IL-6/Jak/STAT and PI3-K/PDK1/Akt about initiation/proliferation/protection in liver regeneration in the following sections.

### ***1. Priming of hepatocytes in liver regeneration***

The restoration of liver volume depends primarily on the proliferation of hepatocytes. Under non-pathological and static conditions, hepatocytes are in a quiescent state (G0 phase) and hardly undergo mitosis responding to growth factors. Once hepatocytes are primed, they show high reactivity to the mitogens[12]. This process largely depends on the interactions between hepatocytes and non-parenchymal cells (Kupffer cells, SECs, HSCs) via cytokines such as TNF-alpha and IL-6. At the beginning of initiation phase of liver regeneration, Kupffer cells are activated by the stimulations such as LPS[13], C3a, C5a[14] and ICAM[15], and then start to produce and secrete TNF-alpha. Kupffer cells express TNF receptor 1 (TNFR1) on their surface

and activate themselves in the autocrine fashion. TNF-alpha/TNFR1 signaling circuit of Kupffer cells is mainly mediated by NF-kB [16]. Because the promoter region of the *IL-6* gene contains an NF-kB binding site, IL-6 also can be produced by the activated Kupffer cells. TNF-alpha and IL-6 secreted by the activated Kupffer cells is possibly involved in the priming process at the initiation phase, which enables hepatocytes to respond to 'proliferation signal' by the growth factors and/or directly to undergo mitosis[12, 17]. These interactions between hepatocytes and non-parenchymal cells (especially Kupffer cells) are mediated by cytokines such as TNF-alpha and IL-6, and achieve the priming process; G0/G1 transition of hepatocytes in other words. IL-6 also plays a lot of other important roles in liver regeneration inducing hepatic production of hepatocyte growth factor (HGF)[17], which acts as a mitogen of hepatocytes and protecting hepatocytes from apoptosis. Deficiency of *IL-6* gene leads to impairment of liver regeneration characterized by liver necrosis and failure[18]. The IL-6 signaling plays a pivotal role in liver regeneration, which is mainly mediated by the mitotic transcription factor, signal transducers and activators of transcription-3 (STAT3) (Fig. 1).

## ***2. Proliferation of hepatocytes in liver regeneration***

After G0/G1 transition in initiation phase, approximately 95% of hepatocytes



will be recruited into the cell cycle[3] and some actually undergo mitosis in the remnant liver tissue. The progression of cell cycle of hepatocytes after PH largely depends on growth factor signaling, especially HGF/Met and EGF family ligands/EGFR pathways.

HGF is a potent growth factor that is mainly derived from activated HSCs and promotes proliferation and DNA synthesis of hepatocytes in a paracrine fashion [19]. HGF is secreted as an inactive precursor, pro-HGF and stored in the extracellular matrix. The activation of HGF is achieved by fibrinolytic system. The Immediate early induction of urokinase-type plasminogen activator receptor (u-PA) after PH initiates the activation of the fibrinolytic cascades and then urokinase-type plasminogen activator (u-PA), plasmin and matrix metalloproteinases (MMPs) are activated. Plasmin and MMPs degrade extracellular matrix (ECM) and release pro-HGF from ECM. Then pro-HGF is cleaved into activated form of HGF by u-PA[20]. And through the suppression of this system, Plasminogen Activator Inhibitor (PAI) and Tissue Inhibitor of MetalloProteinases-1 (TIMP-1) can reduce the efficiency of HGF activation[21]. This post-transcriptional control system can supply sufficient amount of HGF immediately after receiving activation signals. HGF promotes the proliferation of hepatocytes by directly binding to its specific tyrosine kinase type receptor, c-met on the surface of hepatocytes. The HGF/c-met signaling is transmitted to its downstream pathways

including Ras-Raf-MEK, ERK1/2[22], PI3-K/PDK1/Akt[23], and mTOR/S6 kinase pathways. And this contributes to liver regeneration by promoting DNA synthesis, cell cycles progression, cell growth and cell protection.

TGF-alpha is another important growth factor in liver regeneration that directly stimulates DNA synthesis in hepatocytes[24-25]. TGF-alpha belongs to Epithelial Growth Factor (EGF) family that includes EGF, heparin binding EGF-like growth factor and amphiregulin[26]. The members of EGF family share the common receptor EGF receptor (EGFR) and can compensate each other's function. TGF-alpha is secreted by hepatocytes and functions in the autocrine and paracrine fashions. And the production and secretion of TGF-alpha by hepatocytes are promoted by HGF. Similar to c-met, EGFR also promotes cell proliferation and cell growth as a tyrosine kinase receptor (Fig. 1).

### ***3. Progression and maintenance in liver regeneration: roles of cell proliferation and growth in liver regeneration***

Enlargement of liver mass is achieved by 1) cell proliferation that is the increase in number of hepatocytes and 2) cell growth that is the increase in size of hepatocytes. Although the mechanism regulating cell proliferation and growth of hepatocytes remains to be completely elucidated, IL-6/STAT3 pathway and

PI3-K/PDK1/Akt pathway definitely play pivotal roles in regulating proliferation and growth of hepatocytes at least in acute liver response after hepatectomy in rodents, respectively (Fig. 2).

### ***3.1. IL-6/STAT3 signal and hepatocyte proliferation / protection***

IL-6 sends mitotic signals mainly through STAT3 in hepatocytes and plays crucial roles in liver regeneration. The response of STAT3 to PH is regarded as a trigger of liver regeneration because of its rapid response. DNA-binding of STAT3 is observed within 30 minutes and peaks at 3 hours after PH in mice[27]. In *IL-6*-deficient mice, this STAT3 activation is almost suppressed[18]. Therefore, it is considered that the activation of STAT3 is induced exclusively by IL-6 signaling in liver regeneration after PH although STAT3 can be activated by other cytokines such as G-CSF[28] and leptin[29] in other situations.

IL-6/STAT3 signaling pathway is composed of IL-6 receptor, gp130, receptor-associated Janus kinase (Jak) and STAT3. IL-6 receptor forms a complex with two molecules of gp130. After the recognition of IL-6, gp130 immediately transmits IL-6 signal into hepatocytes. gp130 possesses consensus sequence YxxQ motif that is recognized by Src homology 2 (SH2) domain of STAT3 on its cytoplasmic domain[30]. Jak is a phosphorylating enzyme that is responsible for the activation of gp130 and

STAT3. It is bound to the cytoplasmic domain of gp130 in an inactive form and activated by reciprocal phosphorylation after IL-6 binding. STAT3 commonly pre-exists in an inactive form and is activated by Jak. STAT3 consists of five functional domains, amino terminal, coiled-coil domain, DNA binding domain, SH2 domain and transcriptional activation domain[31]. Activated monomers of STAT3 must form dimers to exert their transcriptional function. The dimerization of STAT3 depends only on these reciprocal interactions between the phosphor-tyrosine (Y705) of a monomer of STAT3 and the arginyl residue (R608) in the SH2 pocket of another monomer. The IL-6/STAT3 signaling is transmitted in the following manner[32-34]. The spatial arrangement of IL-6 receptor and gp130 proteins is changed by IL-6 binding to its receptor. Jaks are arranged closer to each other and consequently they phosphorylate and activate their counterparts each other. Activated form of Jak can phosphorylate the tyrosine within the YxxQ motif of gp130. The inactive form of STAT3 is recruited to gp130 by the interaction between phosphor-tyrosine on YxxQ motif of gp130 and SH2 domain of STAT3. Jak induces activation of STAT3 through phosphorylation of tyrosine (Y705). Activated STAT3 is released from gp130 and dimerized through the interaction between its SH2 domain and phosphor-tyrosine on its counterpart. STAT3 dimer then translocates to nucleus and regulates the expression of its target genes (Fig. 3).

The main function of STAT3 in liver regeneration is to promote hepatocyte proliferation. To elucidate the mechanisms by which STAT3 regulates proliferation, liver specific STAT3 deficient mice were created[35], because systemic STAT3 deficient mice were early embryonic lethal[36]. In the liver-specific STAT3 knockout mice, DNA synthesis and mitotic activity of hepatocytes after partial PH is reduced significantly. Among the proteins involved in hepatocyte proliferation, the expressions of cyclin D1 and cyclin E usually occurring within 72 hours after PH is also significantly decreased in liver-specific STAT3 deficient mice. Constitutively dimerized and activated STAT3 (STAT3-C) similarly contributes to analysis of the STAT3 function. Transfection of the *STAT3-C* gene in fibroblasts induces transcription of cyclin D1, Bcl-XL and c-myc[37]. Furthermore, mitogenic effect of STAT3 is demonstrated in rat 20% partial liver transplant model. In this experiment, liver graft transfected with the *Stat3-C* gene during cold preservation showed a greater enlargement of liver graft and hepatocyte proliferation with reduced post-transplant graft damage than the non-treated graft[11].

One of the well investigated mechanisms by which STAT3 promotes liver regeneration is the control of cell cycle progression from G1 to S phase[38]. Cell cycle is considered to be strictly regulated by the periodic expression pattern of cyclins and CDKs. Cell cycle is a sequential process that consists of four conceptual phases, gap 1

phase (G1), synthesis phase (S), gap 2 phase (G2) and mitosis phase (M). Hepatocytes move from quiescent state (G0) into starting point of cell cycle (G1) during the initiation phase, and then they encounter the restriction point in late G1 phase[39]. The expression of G1 cyclins can permit hepatocytes to override the restriction point and enter into S phase. The G1 cyclins include cyclin D and E and the expression of cyclin D1 is regulated by STAT3[35]. Cyclin D1 binds to CDK4/6, which partially phosphorylates Rb protein that binds to E2F to repress its transcriptional activity. Although cyclin D1/CDK4 or 6 complex is not able to derepress E2F completely, partially activated E2F can induce the expression of cyclin E. Then cyclin E forms a complex with CDK2 leading to complete phosphorylation of Rb protein and activation of E2F. Fully activated E2F induces the expression of its target genes that is responsible for transition from G1 to S phase[40-41]. Thus STAT3 regulates G1/S transition of hepatocytes through the control of cyclin D1 expression (Fig 3).

Although apoptosis may be an essential process for the liver to maintain its homeostasis, to prevent hepatic neoplasms and autoimmune diseases and to eliminate viruses, inappropriate apoptotic responses induce tissue destruction and eventually liver dysfunction. In liver surgery including liver transplantation, graft preservation, temporary vascular occlusive maneuver or hemodynamic instability are accompanied by hepatic

ischemia/reperfusion (I/R) and eventually induce apoptosis. These pathological signals are mediated by death receptor pathway, mitochondrial pathway, p53 pathway and MAPK pathway[42]. Apoptosis contributes to pathogenesis and progression in some liver diseases. Hepatitis viruses activate apoptotic signals with immunological mechanisms and/or direct viral effects[43]. Liver damage by HBV infection is alleviated by the blockade of TRAIL pathway[44-45] and HCV promotes apoptosis of hepatocytes by increasing the expression of Fas on hepatocytes and Fas-ligand (FasL) on T cells. And the viral protein such as HBV X protein[46] and HCV core protein[46-47] can induce apoptosis directly interacting with the cytoplasmic domains of death receptors and p53. In fulminant hepatic failure, caspase-3, Fas/FasL and TNF alpha/TNFR is upregulated in liver[48]. Therefore, liver damage (apoptosis of hepatocyte) may progress simultaneously with liver regeneration to some degree, depending on the underlying liver disease.

The target genes of STAT3 include anti-apoptotic genes, *FLIP*, *Bcl-2* and *Bcl-xL* and therefore STAT3 potentially possesses the anti-apoptotic capacity[49]. Apoptosis is initiated by binding of death ligands such as FasL and TRAIL to their specific death receptors. And then caspase-8, -10 and FADD is recruited into the cytoplasmic death domains of death receptors to form the death inducing signaling

complex (DISC) leading to the activation of caspase-8 and -10. FLIP inhibits this recruitment of caspase-8 and-10 competitively[50]. Cleaved and activated caspase-8 can propagate its signal directly to caspase-3 or through mitochondrial pathway mediated by BH3 only proteins such as Bid[51]. Then apoptotic proteins, Bax and Bak induces mitochondrial outer membrane permeabilization (MOMP) and anti-apoptotic proteins, Bcl-2 and Bcl-xL impair this step[52]. Interestingly, STAT3 is also known to possess anti-oxidative capacity. Hypoxia/reoxygenation (H/R) and I/R can induce apoptosis of hepatocytes in a redox-dependent manner, where generation of reactive oxygen species (ROS) in hepatocytes will activate redox-sensitive caspases such as caspase-3/-9. STAT3 also upregulates genes of *Ref-1*[53] and *Mn-SOD*[54] that protects hepatocytes from ROS-mediated apoptotic cell death (Fig 4).

As mentioned above, STAT3 largely contributes to liver regeneration through its cell proliferative and protective properties. However, it may possibly impair liver regeneration inversely in some situations. Fatty liver (steatosis of hepatocytes) is a known risk factor of liver surgery such as PH[55-56], liver transplantation[57-59] and donor operation[60-61]. The impairment of hepatocyte proliferation is thought to be due to the effect of steatosis in part. In steatotic liver, cell cycle-related proliferative disorders occur at mid-S phase. Reduced expression of Wee1 and Myt1 kinases may



maintain Cdc2 in an unphosphorylated state and block cell cycle progression in mid-S phase[62]. Some researchers reported that in ob/ob (or db/db) mice with fatty liver, a higher level of phospho-STAT3 was observed than normal mice. And PH phosphorylated STAT3 far more in these mice than normal mice. Despite STAT3 was markedly phosphorylated, DNA synthesis and transcription of cyclin D1 in ob/ob mice liver remained at lower level. It seems strange that STAT3 activation was positively correlated with expression of p21[63]. p21 is a CDK inhibitor (CKI) protein that inhibits G1/S progression binding to cyclin D1/CDK4 of 6 complex in hepatocytes[64]. *p21* gene, upregulated by STAT3, may impair proliferation of hepatocytes in some situations[65], or inversely may potentially afford scaffolding effect for STAT3-mediated cell proliferation.

As such, the biological significance of STAT3 is still unclear at present. The functions of STAT3 and its responsiveness, however, are definitely regulated by the patho-physiological situations that the liver is suffering. They may be affected by the metabolic states (such as steatosis and metabolic syndrome), age and/or redox states (Fig 3).

### ***3.2. PI3-K/PDK1/Akt pathway and hepatocyte growth***

PI3-K/PDK1/Akt pathway has been known as a survival pathway functioning

in anti-apoptosis[66-68], anti-oxidation[69-70] and protein synthesis. Recently, it was revealed that PI3-K/PDK1/Akt pathway is responsible for regulating cell growth and determining cell size / functions[71-76]. PI3-K/PDK1/Akt signals are initiated by the activation of the receptor tyrosine kinases (RTKs) or receptors coupled with G proteins by IL-6[77], TNF-alpha[78-79], HGF[23], EGF[80-81], TGF-alpha[81] or many other signaling molecules.

PI3-K is classified in three categories, class I, II and III[82] by their structure. Class I PI3-Ks are kinases composed of a catalytic subunit of p110 and an adaptor/regulatory subunit of p85. PI3-K exclusively phosphorylate phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2) to form phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P3) in vivo [83-84]. This phosphorylation capacity of PI3-K is negated by phosphatase and tensin homolog deleted on chromosome 10 (PTEN) that degrades PI-3,4,5-P3 to PI-4,5-P2 conversely[85]. PI-3,4,5-P3 provides a phospholipid binding substrates for PDK1 and Akt, and recruits them to the site on the membrane where the PI-3,4,5-P3 is fixed[86].

Akt, a down-stream molecule of PI3-K/PDK1, is a serine/threonine kinase that consists of a kinase domain, a hydrophobic domain and a pleckstrin homology domain which possesses the affinity for PI-3,4-P2 and PI-3,4,5-P3. Akt can be activated by

phosphorylation at two sites, threonine residue (T308) in the activation loop of kinase domain, and serine residue (S473) in the hydrophobic domain. Although phosphorylation at T308 is sufficient for the activation of Akt, full activation needs the phosphorylation of both T308 and S473[87-90]. PDK1 mainly activates Akt by phosphorylating threonine residue at 308[91], and PDK2, not determined yet, phosphorylates Akt at serine.

Mammalian target of rapamycin (mTOR) is one of the important downstream molecules of Akt for cell growth[92]. The activation of mTOR is promoted by Akt in a suppressive manner via TSC1, TSC2 and Rheb suppression. Akt phosphorylates TSC2 to inhibit function of the TSC1-TSC2 complex and then to inhibit Rheb function. Rheb positively modulates the function of mTOR[93]. mTOR suppresses and activates eIF4E inhibiting 4E-BP1 activity and it also activates S6K1 and S6K2. These downstream effectors of mTOR regulate protein synthesis, cell size, cell cycle progression, glucose homeostasis and survival of newborn[94]. And PDK1 can activate S6K1 and S6K2 directly in addition to the activation of Akt (Fig. 5).

We previously reported that PI3-K/PDK1/Akt pathway coexists with STAT3 pathway and can compensate for the insufficiency of its counterpart in some specified conditions during liver regeneration. In liver specific *STAT3*-knockout mice, no

significant difference was observed in macroscopic liver regeneration, compared to the control mice. Although the expression of cyclin D1 and therefore mitosis of hepatocyte were definitely impaired, the liver size of *STAT3*-knockout mice 3 and 14 days post-70% PH was almost equal to those of normal mice. This strange observation about liver regeneration was explained by the enlarged size of each hepatocyte. The cell size of hepatocyte in *STAT3*-knockout liver was significantly larger than that in normal liver 72 hours post-PH. And in 14 days after PH, the cell size of *STAT3*-knockout liver was normalized. Along with the enlarged cell size (cell growth), the marked phosphorylation of Akt and its downstream molecules, p70<sup>S6K</sup>, mTOR and GSK-3 beta was observed[95]. To elucidate the mechanism of ‘cell size regulation’ during liver regeneration, we next generated liver specific *PDK1*-knockout mice. In *PDK1*-knockout mice, phosphorylation of threonine residue (T308) of Akt was impaired and therefore the phosphorylation/activation of p70<sup>S6K</sup>, mTOR was decreased. These *PDK1*-knockout mice couldn’t endure 70% PH, and macroscopic liver regeneration 3 and 14 days after 30% PH was severely impaired. This phenotype was normalized by transfection of the adenovirus vector encoding PDK1 interacting fragment (pif) pocket mutant of *Pdk1* that signals exclusively to Akt but not to p70<sup>S6K</sup> or any others[96-97]. Based on these observations, PDK1 was evidenced to contribute to early phase of liver regeneration

promoting cell growth via Akt activation. These experiments provided another novel insight to the understandings of molecular/cellular mechanisms in liver regeneration.

There are some other reports supporting the importance of PI3-K/PDK1/Akt-mediated growth in liver. Stiles *et al.* reported that liver-specific deletion of *PTEN* induced marked phosphorylation of Akt, where significant hepatomegaly was observed with increased size of cell size[98]. Mullany *et al.* also demonstrated the cell growth induced by Akt. They transiently over-expressed Akt and cyclinD1 in the liver of mice by transfecting an adenovirus vector. While the transfection of *cyclinD1* promoted DNA synthesis/cell proliferation and induced liver growth, far tremendous liver growth was observed in *Akt*-transfected mice. In the *Akt*-transfected liver, DNA synthesis was observed similar to the control liver, but the evidently increased size of liver cells were confirmed in flow cytometrical analysis[99]. Jackson *et al.* reported that inhibition of PI3-K leads to significant decrease in hepatocyte proliferation, especially at the earliest time points. They used wortmannin and siRNA targeting *p85 alpha* and *p110 alpha* to inhibit PI3-K activity. Liver regeneration in early period after hepatectomy was impaired significantly in the mice pre-treated with siRNA and wortmannin 24 hours prior to PH. In the PI3-K-inhibited liver, the aggregation of Kupffer cells after PH was reduced and the serum levels of

cytokines, IL-6 and TNF-alpha were also decreased. They uniquely concluded that PI3-K contributes to liver regeneration especially early after PH by promoting migration of Kupffer cells to the site of regeneration, priming of hepatocytes with cytokines (IL-6 and TNF-alpha) and DNA synthesis of hepatocytes[100]. The above observation may support our observation on PI3-K/STAT3 axis that PI3-K directly sends a mitogenic signal to STAT3 and promotes hepatocyte proliferation directly or indirectly.

### *Conclusion*

Although the precise mechanisms of liver regeneration are still unknown, the accumulation of evidences prompted us to study and understand the mechanisms of impaired regeneration in the diseased liver from the aspect of molecular biology. As some mechanisms are presumed to be responsible for the impairment of regeneration in steatotic liver, it is suggested that some of aberrant behavior of biological molecules cause liver dysfunction.

Liver regeneration has come to be regarded as an integration of multiple separated processes, including cell proliferation and growth. Here, we propose the schematic mechanisms of molecular regulation of liver regeneration, focusing on IL-6/STAT3 and PI3-K/PDK1/Akt pathways in hepatocytes (Fig. 6). Because the liver is

composed of mortal cells, the liver is in a state of dynamic equilibrium even when it is in quiescent state. Therefore, cell death may play an important role in regulation of liver regeneration especially termination phase. The studies on cell death and liver regeneration have just begun but the cell death may be the third piece of understanding liver regeneration hereafter.

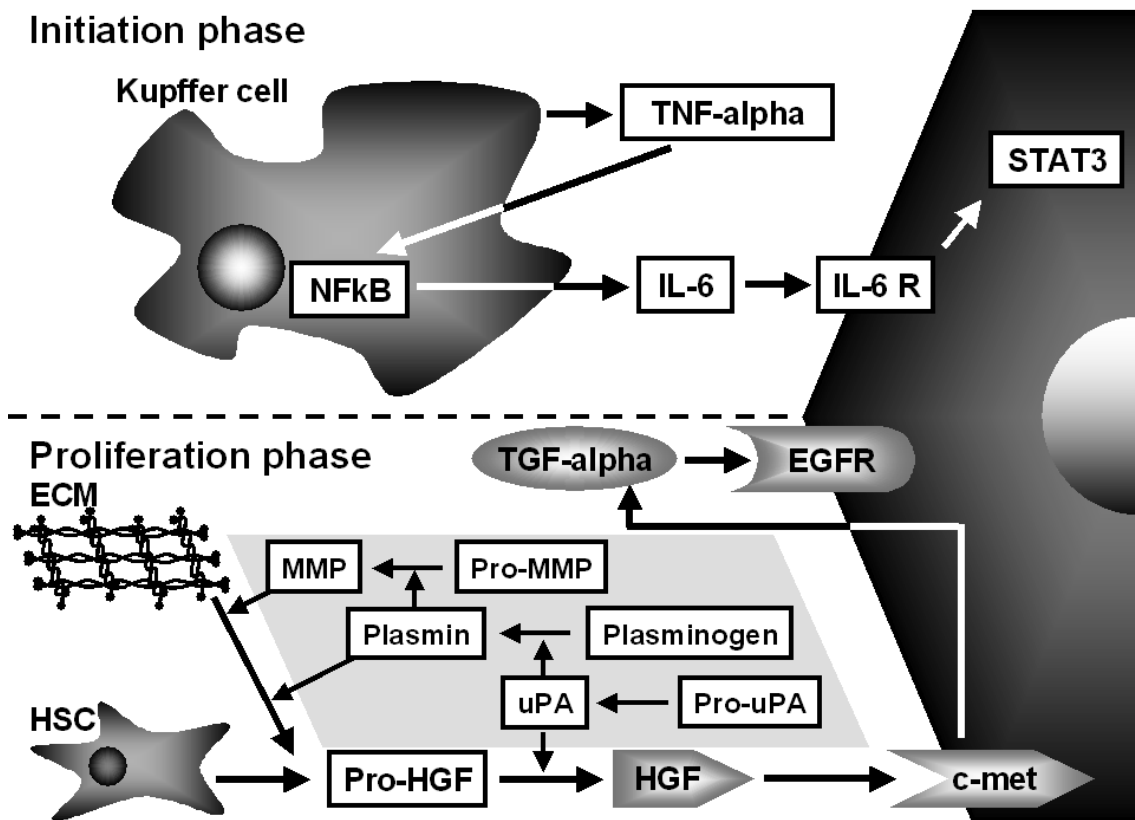


Fig. 1 Intercellular interactions and initiation/proliferation in liver regeneration

Kupffer cells primes hepatocytes via cytokines such as TNF-alpha and IL-6 (initiation phase). Then HGF released from HSC and ECM and activated by fibrinolytic system, leads to proliferation of hepatocytes (proliferation phase).



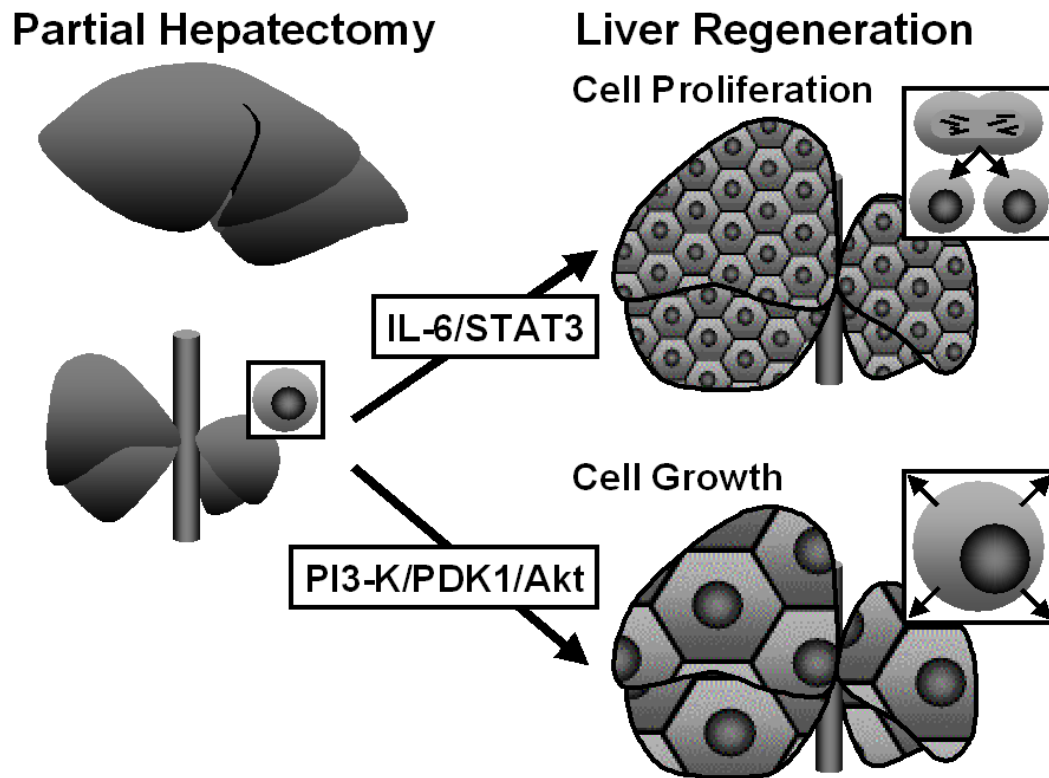


Fig. 2 Cell proliferation and cell growth in liver regeneration

Liver regeneration is basically achieved by the combination of two distinct manners, cell proliferation and growth. Cell proliferation (increase in number of hepatocytes) is mediated by IL-6/STAT3 pathway and cell growth (increase in size of hepatocytes) by PI3-K/PDK/Akt pathway.

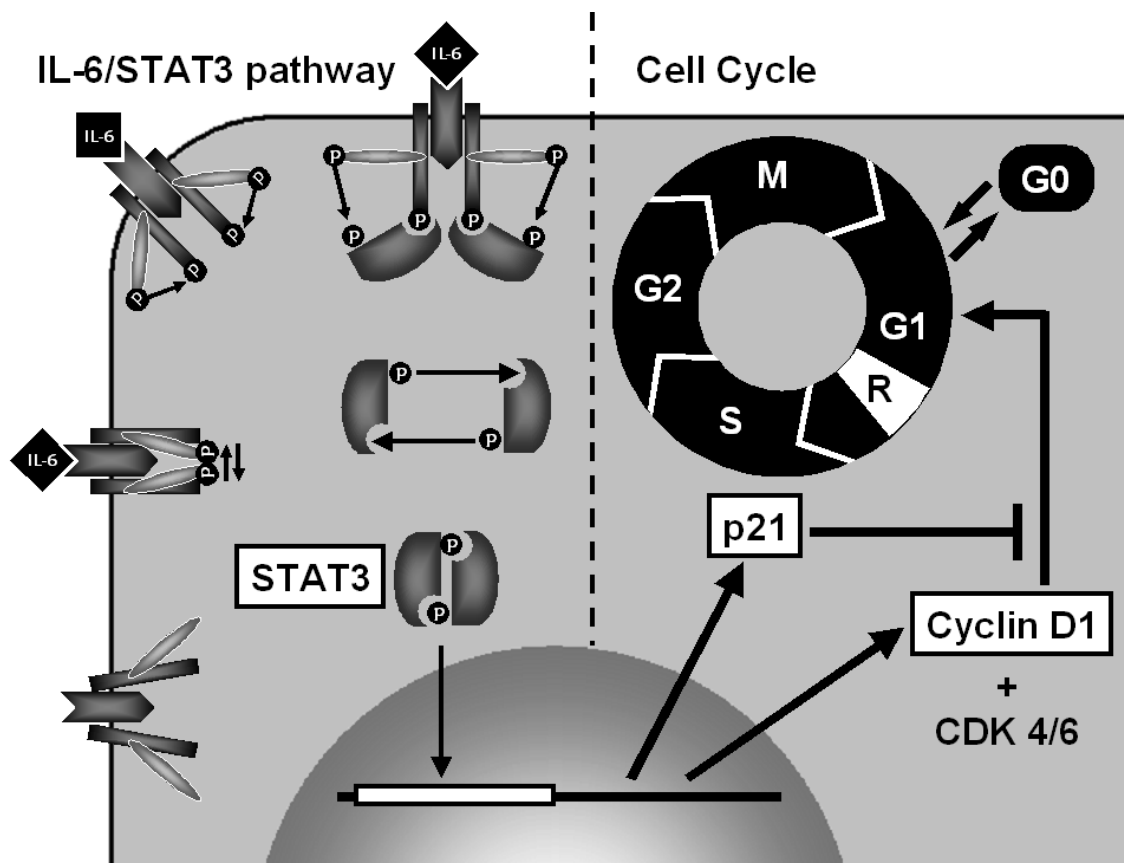


Fig. 3 IL-6/Jak/STAT3 pathway and cell proliferation

STAT3 is activated by IL-6 signal through Jak/STAT pathway in hepatocytes. STAT3 promotes the expressions of cyclin D1 and p21 to control the progression of cell cycle.

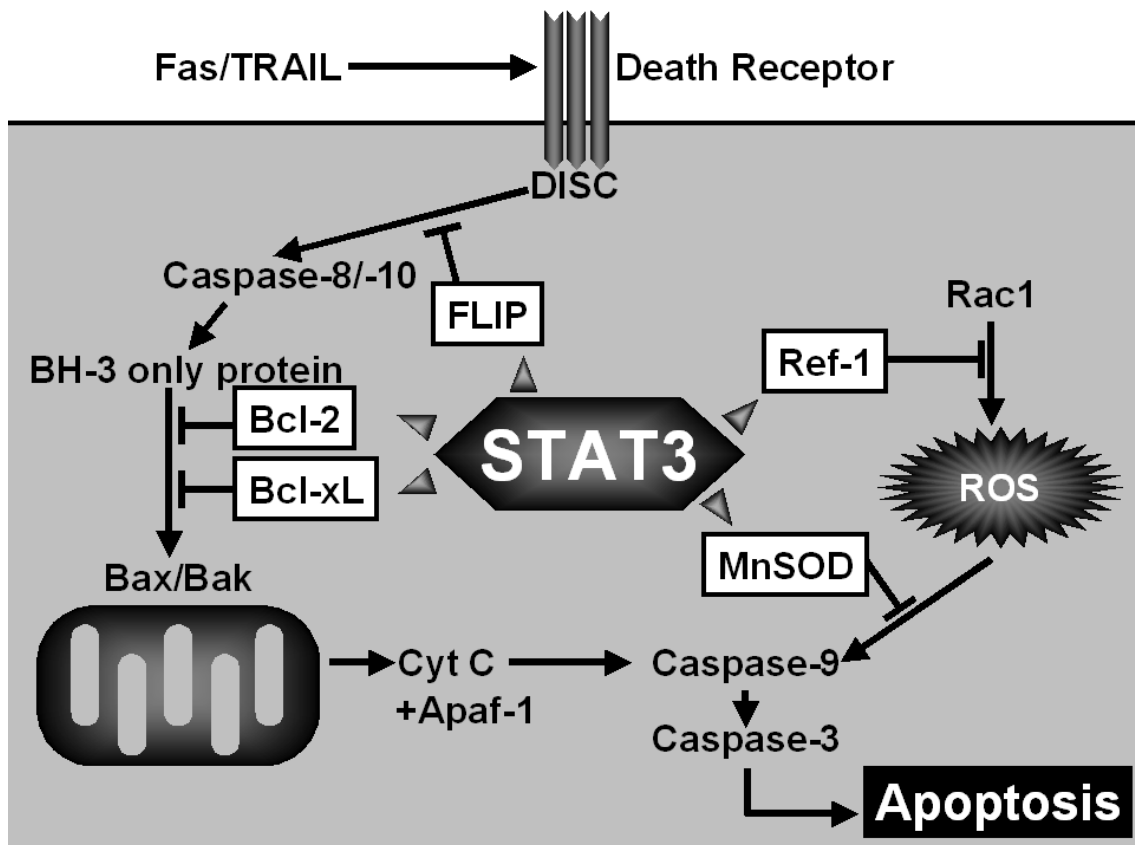


Fig. 4 Jak/STAT and anti-apoptosis/-oxidation

STAT3 protects hepatocytes from cell death by inducing anti-apoptotic proteins (FLIP, Bcl-2 and Bcl-xL) and anti-oxidation proteins (Ref-1 and MnSOD).

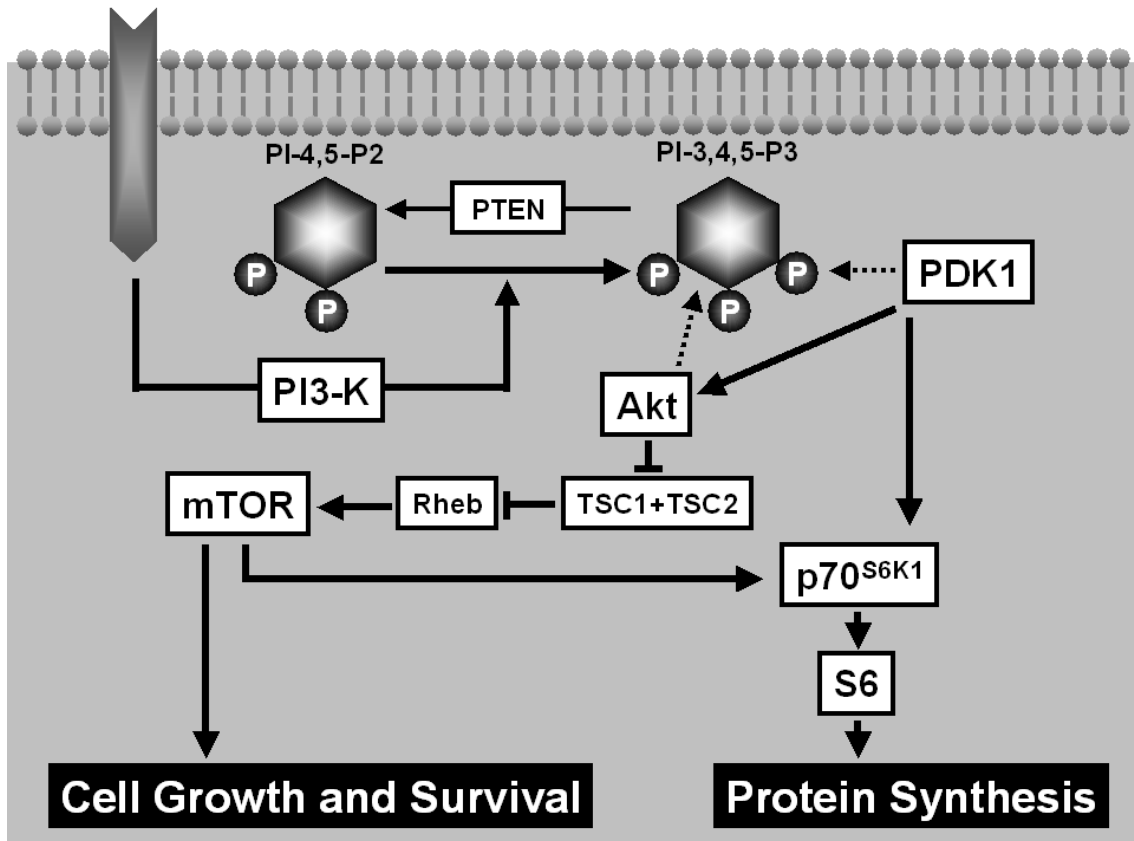


Fig. 5 PI3-K/PDK1/Akt and mTOR for cell growth, survival and protein synthesis

PI3-K/PDK1/Akt pathway is responsible for the determination of sizes of hepatocytes through its downstream effectors (mTOR).

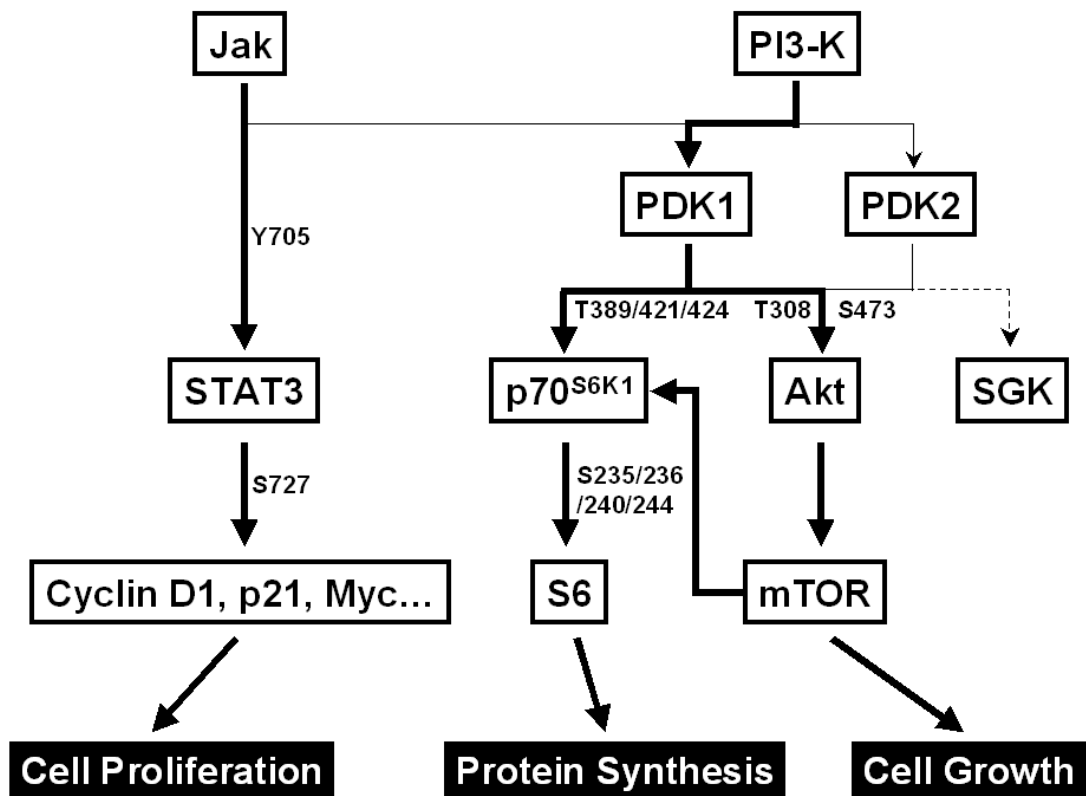


Fig. 6 Two major signaling pathways in liver regeneration: IL-6/Jak/STAT3 and PI3-K/PDK1/Akt for cell growth, survival and protein synthesis.

IL-6/Jak/STAT3 and PI3-K/PDK1/Akt pathways play important roles to achieve multiple processes required for liver regenerations (cell proliferation, growth and protein synthesis).

## References

1. Viebahn CS, Yeoh GC. What fires prometheus? The link between inflammation and regeneration following chronic liver injury. *Int J Biochem Cell Biol.* 2008;40(5):855-73.
2. Pahlavan PS, Feldmann RE, Jr., Zavos C, Kountouras J. Prometheus' challenge: molecular, cellular and systemic aspects of liver regeneration. *J Surg Res.* 2006 Aug;134(2):238-51.
3. Taub R. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol.* 2004 Oct;5(10):836-47.
4. Moro L, Marra E, Capuano F, Greco M. Thyroid hormone treatment of hypothyroid rats restores the regenerative capacity and the mitochondrial membrane permeability properties of the liver after partial hepatectomy. *Endocrinology.* 2004 Nov;145(11):5121-8.
5. Malik R, Habib M, Tootle R, Hodgson H. Exogenous thyroid hormone induces liver enlargement, whilst maintaining regenerative potential--a study relevant to donor preconditioning. *Am J Transplant.* 2005 Aug;5(8):1801-7.
6. Kopplow K, Wayss K, Enzmann H, Mayer D. Dehydroepiandrosterone causes hyperplasia and impairs regeneration in rat liver. *Int J Oncol.* 2005 Dec;27(6):1551-8.
7. Sabugal R, Robert MQ, Julve J, Auwerx J, Llobera M, Peinado-Onsurbe J. Hepatic regeneration induces changes in lipoprotein lipase activity in several tissues and its re-expression in the liver. *Biochem J.* 1996 Sep 1;318 ( Pt 2):597-602.
8. Tang TX, Hashimoto T, Chao LY, Itoh K, Manabe T. Effects of partial pancreatectomy on liver regeneration in rats. *J Surg Res.* 1997 Sep;72(1):8-14.
9. Marti U, Burwen SJ, Jones AL. Biological effects of epidermal growth factor, with emphasis on the gastrointestinal tract and liver: an update. *Hepatology.* 1989 Jan;9(1):126-38.
10. Kiba T. The role of the autonomic nervous system in liver regeneration and apoptosis--recent developments. *Digestion.* 2002;66(2):79-88.
11. Huda KA, Guo L, Haga S, Murata H, Ogino T, Fukai M, et al. Ex vivo adenoviral gene transfer of constitutively activated STAT3 reduces post-transplant liver injury and promotes regeneration in a 20% rat partial liver transplant model. *Transpl Int.* 2006 May;19(5):415-23.
12. Webber EM, Bruix J, Pierce RH, Fausto N. Tumor necrosis factor primes hepatocytes for DNA replication in the rat. *Hepatology.* 1998 Nov;28(5):1226-34.
13. Cornell RP, Liljequist BL, Bartizal KF. Depressed liver regeneration after partial hepatectomy of germ-free, athymic and lipopolysaccharide-resistant mice. *Hepatology.* 1990 Jun;11(6):916-22.

14. Strey CW, Markiewski M, Mastellos D, Tudoran R, Spruce LA, Greenbaum LE, et al. The proinflammatory mediators C3a and C5a are essential for liver regeneration. *J Exp Med*. 2003 Sep 15;198(6):913-23.
15. Selzner N, Selzner M, Odermatt B, Tian Y, Van Rooijen N, Clavien PA. ICAM-1 triggers liver regeneration through leukocyte recruitment and Kupffer cell-dependent release of TNF-alpha/IL-6 in mice. *Gastroenterology*. 2003 Mar;124(3):692-700.
16. Libermann TA, Baltimore D. Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. *Mol Cell Biol*. 1990 May;10(5):2327-34.
17. Zimmers TA, McKillop IH, Pierce RH, Yoo JY, Koniaris LG. Massive liver growth in mice induced by systemic interleukin 6 administration. *Hepatology*. 2003 Aug;38(2):326-34.
18. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, et al. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science*. 1996 Nov 22;274(5291):1379-83.
19. Huh CG, Factor VM, Sanchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. *Proc Natl Acad Sci U S A*. 2004 Mar 30;101(13):4477-82.
20. Kim TH, Mars WM, Stolz DB, Petersen BE, Michalopoulos GK. Extracellular matrix remodeling at the early stages of liver regeneration in the rat. *Hepatology*. 1997 Oct;26(4):896-904.
21. Mohammed FF, Pennington CJ, Kassiri Z, Rubin JS, Soloway PD, Ruther U, et al. Metalloproteinase inhibitor TIMP-1 affects hepatocyte cell cycle via HGF activation in murine liver regeneration. *Hepatology*. 2005 Apr;41(4):857-67.
22. Borowiak M, Garratt AN, Wustefeld T, Strehle M, Trautwein C, Birchmeier C. Met provides essential signals for liver regeneration. *Proc Natl Acad Sci U S A*. 2004 Jul 20;101(29):10608-13.
23. Okano J, Shiota G, Matsumoto K, Yasui S, Kurimasa A, Hisatome I, et al. Hepatocyte growth factor exerts a proliferative effect on oval cells through the PI3K/AKT signaling pathway. *Biochem Biophys Res Commun*. 2003 Sep 19;309(2):298-304.
24. Scheving LA, Stevenson MC, Taylormoore JM, Traxler P, Russell WE. Integral role of the EGF receptor in HGF-mediated hepatocyte proliferation. *Biochem Biophys Res Commun*. 2002 Jan 11;290(1):197-203.
25. Tomiya T, Ogata I, Yamaoka M, Yanase M, Inoue Y, Fujiwara K. The mitogenic activity of hepatocyte growth factor on rat hepatocytes is dependent upon endogenous transforming growth factor-alpha. *Am J Pathol*. 2000 Nov;157(5):1693-701.
26. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology*. 2006 Feb;43(2)

Suppl 1):S45-53.

27. Cressman DE, Diamond RH, Taub R. Rapid activation of the Stat3 transcription complex in liver regeneration. *Hepatology*. 1995 May;21(5):1443-9.
28. Akira S. Roles of STAT3 defined by tissue-specific gene targeting. *Oncogene*. 2000 May 15;19(21):2607-11.
29. Waelput W, Verhee A, Broekaert D, Eyckerman S, Vandekerckhove J, Beattie JH, et al. Identification and expression analysis of leptin-regulated immediate early response and late target genes. *Biochem J*. 2000 May 15;348 Pt 1:55-61.
30. Levy DE, Lee CK. What does Stat3 do? *J Clin Invest*. 2002 May;109(9):1143-8.
31. Schindler CW. Series introduction. JAK-STAT signaling in human disease. *J Clin Invest*. 2002 May;109(9):1133-7.
32. Darnell JE, Jr., Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*. 1994 Jun 3;264(5164):1415-21.
33. Darnell JE, Jr. STATs and gene regulation. *Science*. 1997 Sep 12;277(5332):1630-5.
34. Bromberg J, Darnell JE, Jr. The role of STATs in transcriptional control and their impact on cellular function. *Oncogene*. 2000 May 15;19(21):2468-73.
35. Li W, Liang X, Kellendonk C, Poli V, Taub R. STAT3 contributes to the mitogenic response of hepatocytes during liver regeneration. *J Biol Chem*. 2002 Aug 9;277(32):28411-7.
36. Takeda K, Noguchi K, Shi W, Tanaka T, Matsumoto M, Yoshida N, et al. Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. *Proc Natl Acad Sci U S A*. 1997 Apr 15;94(8):3801-4.
37. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, et al. Stat3 as an oncogene. *Cell*. 1999 Aug 6;98(3):295-303.
38. Terui K, Ozaki M. The role of STAT3 in liver regeneration. *Drugs Today (Barc)*. 2005 Jul;41(7):461-9.
39. Loyer P, Cariou S, Glaise D, Bilodeau M, Baffet G, Guguen-Guillouzo C. Growth factor dependence of progression through G1 and S phases of adult rat hepatocytes in vitro. Evidence of a mitogen restriction point in mid-late G1. *J Biol Chem*. 1996 May 10;271(19):11484-92.
40. Lavia P, Jansen-Durr P. E2F target genes and cell-cycle checkpoint control. *Bioessays*. 1999 Mar;21(3):221-30.
41. Takahashi-Yanaga F, Sasaguri T. GSK-3beta regulates cyclin D1 expression: a new target for chemotherapy. *Cell Signal*. 2008 Apr;20(4):581-9.
42. Georgiev P, Dahm F, Graf R, Clavien PA. Blocking the path to death:



anti-apoptotic molecules in ischemia/reperfusion injury of the liver. *Curr Pharm Des.* 2006;12(23):2911-21.

43. Clarke P, Tyler KL. Apoptosis in animal models of virus-induced disease. *Nat Rev Microbiol.* 2009 Feb;7(2):144-55.

44. Liu YG, Liu SX, Liang XH, Zhang Q, Gao LF, Han LH, et al. Blockade of TRAIL pathway ameliorates HBV-induced hepatocyte apoptosis in an acute hepatitis model. *Biochem Biophys Res Commun.* 2007 Jan 12;352(2):329-34.

45. Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science.* 1997 Aug 8;277(5327):818-21.

46. Boya P, Pauleau AL, Poncet D, Gonzalez-Polo RA, Zamzami N, Kroemer G. Viral proteins targeting mitochondria: controlling cell death. *Biochim Biophys Acta.* 2004 Dec 6;1659(2-3):178-89.

47. Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, et al. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology.* 2002 Feb;122(2):366-75.

48. Leifeld L, Nattermann J, Fielenbach M, Schmitz V, Sauerbruch T, Spengler U. Intrahepatic activation of caspases in human fulminant hepatic failure. *Liver Int.* 2006 Sep;26(7):872-9.

49. Kovalovich K, Li W, DeAngelis R, Greenbaum LE, Ciliberto G, Taub R. Interleukin-6 protects against Fas-mediated death by establishing a critical level of anti-apoptotic hepatic proteins FLIP, Bcl-2, and Bcl-xL. *J Biol Chem.* 2001 Jul 13;276(28):26605-13.

50. Pennarun B, Meijer A, de Vries EG, Kleibeuker JH, Kruyt F, de Jong S. Playing the DISC: Turning on TRAIL death receptor-mediated apoptosis in cancer. *Biochim Biophys Acta.* 2009 Dec 2.

51. Kruidering M, Evan GI. Caspase-8 in apoptosis: the beginning of "the end"? *IUBMB Life.* 2000 Aug;50(2):85-90.

52. Chipuk JE, Green DR. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? *Trends Cell Biol.* 2008 Apr;18(4):157-64.

53. Haga S, Terui K, Zhang HQ, Enosawa S, Ogawa W, Inoue H, et al. Stat3 protects against Fas-induced liver injury by redox-dependent and -independent mechanisms. *J Clin Invest.* 2003 Oct;112(7):989-98.

54. Terui K, Enosawa S, Haga S, Zhang HQ, Kuroda H, Kouchi K, et al. Stat3 confers resistance against hypoxia/reoxygenation-induced oxidative injury in hepatocytes through upregulation of Mn-SOD. *J Hepatol.* 2004 Dec;41(6):957-65.

55. Vetelainen R, van Vliet A, Gouma DJ, van Gulik TM. Steatosis as a risk factor in liver surgery. *Ann Surg.* 2007 Jan;245(1):20-30.
56. Kooby DA, Fong Y, Suriawinata A, Gonen M, Allen PJ, Klimstra DS, et al. Impact of steatosis on perioperative outcome following hepatic resection. *J Gastrointest Surg.* 2003 Dec;7(8):1034-44.
57. Sauer P, Schemmer P, Uhl W, Encke J. Living-donor liver transplantation: evaluation of donor and recipient. *Nephrol Dial Transplant.* 2004 Jul;19 Suppl 4:iv11-5.
58. Imber CJ, St Peter SD, Handa A, Friend PJ. Hepatic steatosis and its relationship to transplantation. *Liver Transpl.* 2002 May;8(5):415-23.
59. Crowley H, Lewis WD, Gordon F, Jenkins R, Khettry U. Steatosis in donor and transplant liver biopsies. *Hum Pathol.* 2000 Oct;31(10):1209-13.
60. Akabayashi A, Slingsby BT, Fujita M. The first donor death after living-related liver transplantation in Japan. *Transplantation.* 2004 Feb 27;77(4):634.
61. Yamamoto K, Takada Y, Fujimoto Y, Haga H, Oike F, Kobayashi N, et al. Nonalcoholic steatohepatitis in donors for living donor liver transplantation. *Transplantation.* 2007 Feb 15;83(3):257-62.
62. Murata H, Yagi T, Iwagaki H, Ogino T, Sadamori H, Matsukawa H, et al. Mechanism of impaired regeneration of fatty liver in mouse partial hepatectomy model. *J Gastroenterol Hepatol.* 2007 Dec;22(12):2173-80.
63. Torbenson M, Yang SQ, Liu HZ, Huang J, Gage W, Diehl AM. STAT-3 overexpression and p21 up-regulation accompany impaired regeneration of fatty livers. *Am J Pathol.* 2002 Jul;161(1):155-61.
64. Wu H, Wade M, Krall L, Grisham J, Xiong Y, Van Dyke T. Targeted in vivo expression of the cyclin-dependent kinase inhibitor p21 halts hepatocyte cell-cycle progression, postnatal liver development and regeneration. *Genes Dev.* 1996 Feb 1;10(3):245-60.
65. Chin YE, Kitagawa M, Su WC, You ZH, Iwamoto Y, Fu XY. Cell growth arrest and induction of cyclin-dependent kinase inhibitor p21 WAF1/CIP1 mediated by STAT1. *Science.* 1996 May 3;272(5262):719-22.
66. Tanaka Y, Gavrielides MV, Mitsuuchi Y, Fujii T, Kazanietz MG. Protein kinase C promotes apoptosis in LNCaP prostate cancer cells through activation of p38 MAPK and inhibition of the Akt survival pathway. *J Biol Chem.* 2003 Sep 5;278(36):33753-62.
67. Conery AR, Cao Y, Thompson EA, Townsend CM, Jr., Ko TC, Luo K. Akt interacts directly with Smad3 to regulate the sensitivity to TGF-beta induced apoptosis. *Nat Cell Biol.* 2004 Apr;6(4):366-72.
68. Gardai SJ, Hildeman DA, Frankel SK, Whitlock BB, Frasca SC, Borregaard N, et

- al. Phosphorylation of Bax Ser184 by Akt regulates its activity and apoptosis in neutrophils. *J Biol Chem*. 2004 May 14;279(20):21085-95.
69. Lu Y, Parkyn L, Otterbein LE, Kureishi Y, Walsh K, Ray A, et al. Activated Akt protects the lung from oxidant-induced injury and delays death of mice. *J Exp Med*. 2001 Feb 19;193(4):545-49.
70. Ozaki M, Haga S, Zhang HQ, Irani K, Suzuki S. Inhibition of hypoxia/reoxygenation-induced oxidative stress in HGF-stimulated antiapoptotic signaling: role of PI3-K and Akt kinase upon rac1. *Cell Death Differ*. 2003 May;10(5):508-15.
71. Pende M, Kozma SC, Jaquet M, Oorschot V, Burcelin R, Le Marchand-Brustel Y, et al. Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. *Nature*. 2000 Dec 21-28;408(6815):994-7.
72. Edinger AL, Thompson CB. Akt maintains cell size and survival by increasing mTOR-dependent nutrient uptake. *Mol Biol Cell*. 2002 Jul;13(7):2276-88.
73. M GA, Uddin S, Mahmud D, Damacela I, Lavelle D, Ahmed M, et al. Regulation of myeloma cell growth through Akt/Gsk3/forkhead signaling pathway. *Biochem Biophys Res Commun*. 2002 Oct 4;297(4):760-4.
74. Faridi J, Fawcett J, Wang L, Roth RA. Akt promotes increased mammalian cell size by stimulating protein synthesis and inhibiting protein degradation. *Am J Physiol Endocrinol Metab*. 2003 Nov;285(5):E964-72.
75. Latronico MV, Costinean S, Lavitrano ML, Peschle C, Condorelli G. Regulation of cell size and contractile function by AKT in cardiomyocytes. *Ann N Y Acad Sci*. 2004 May;1015:250-60.
76. Mourani PM, Garl PJ, Wenzlau JM, Carpenter TC, Stenmark KR, Weiser-Evans MC. Unique, highly proliferative growth phenotype expressed by embryonic and neointimal smooth muscle cells is driven by constitutive Akt, mTOR, and p70S6K signaling and is actively repressed by PTEN. *Circulation*. 2004 Mar 16;109(10):1299-306.
77. Desmots F, Rissel M, Gilot D, Lagadic-Gossmann D, Morel F, Guguen-Guillouzo C, et al. Pro-inflammatory cytokines tumor necrosis factor alpha and interleukin-6 and survival factor epidermal growth factor positively regulate the murine GSTA4 enzyme in hepatocytes. *J Biol Chem*. 2002 May 17;277(20):17892-900.
78. Osawa Y, Banno Y, Nagaki M, Brenner DA, Naiki T, Nozawa Y, et al. TNF-alpha-induced sphingosine 1-phosphate inhibits apoptosis through a phosphatidylinositol 3-kinase/Akt pathway in human hepatocytes. *J Immunol*. 2001 Jul 1;167(1):173-80.
79. Puri KD, Doggett TA, Huang CY, Douangpanya J, Hayflick JS, Turner M, et al. The role of endothelial PI3Kgamma activity in neutrophil trafficking. *Blood*. 2005 Jul

1;106(1):150-7.

80. Munugalavadla V, Borneo J, Ingram DA, Kapur R. p85alpha subunit of class IA PI-3 kinase is crucial for macrophage growth and migration. *Blood*. 2005 Jul 1;106(1):103-9.
81. Koniaris LG, McKillop IH, Schwartz SI, Zimmers TA. Liver regeneration. *J Am Coll Surg*. 2003 Oct;197(4):634-59.
82. Vanhaesebroeck B, Waterfield MD. Signaling by distinct classes of phosphoinositide 3-kinases. *Exp Cell Res*. 1999 Nov 25;253(1):239-54.
83. Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, Gonzalez-Baron M. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev*. 2004 Apr;30(2):193-204.
84. Duronio V. The life of a cell: apoptosis regulation by the PI3K/PKB pathway. *Biochem J*. 2008 Nov 1;415(3):333-44.
85. Oudit GY, Sun H, Kerfant BG, Crackower MA, Penninger JM, Backx PH. The role of phosphoinositide-3 kinase and PTEN in cardiovascular physiology and disease. *J Mol Cell Cardiol*. 2004 Aug;37(2):449-71.
86. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, et al. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. *Curr Biol*. 1997 Apr 1;7(4):261-9.
87. Andjelkovic M, Alessi DR, Meier R, Fernandez A, Lamb NJ, Frech M, et al. Role of translocation in the activation and function of protein kinase B. *J Biol Chem*. 1997 Dec 12;272(50):31515-24.
88. Franke TF, Kaplan DR, Cantley LC, Toker A. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. *Science*. 1997 Jan 31;275(5300):665-8.
89. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, et al. Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J*. 1996 Dec 2;15(23):6541-51.
90. Matheny RW, Jr., Adamo ML. Current perspectives on Akt activation and Akt-ions. *Exp Biol Med (Maywood)*. 2009 Nov;234(11):1264-70.
91. Stokoe D, Stephens LR, Copeland T, Gaffney PR, Reese CB, Painter GF, et al. Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. *Science*. 1997 Jul 25;277(5325):567-70.
92. Fingar DC, Salama S, Tsou C, Harlow E, Blenis J. Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes Dev*. 2002 Jun 15;16(12):1472-87.
93. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev*. 2004 Aug 15;18(16):1926-45.

94. Ruvinsky I, Meyuhas O. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. *Trends Biochem Sci.* 2006 Jun;31(6):342-8.
95. Haga S, Ogawa W, Inoue H, Terui K, Ogino T, Igarashi R, et al. Compensatory recovery of liver mass by Akt-mediated hepatocellular hypertrophy in liver-specific STAT3-deficient mice. *J Hepatol.* 2005 Nov;43(5):799-807.
96. Collins BJ, Deak M, Arthur JS, Armit LJ, Alessi DR. In vivo role of the PIF-binding docking site of PDK1 defined by knock-in mutation. *EMBO J.* 2003 Aug 15;22(16):4202-11.
97. Haga S, Ozaki M, Inoue H, Okamoto Y, Ogawa W, Takeda K, et al. The survival pathways phosphatidylinositol-3 kinase (PI3-K)/phosphoinositide-dependent protein kinase 1 (PDK1)/Akt modulate liver regeneration through hepatocyte size rather than proliferation. *Hepatology.* 2009 Jan;49(1):204-14.
98. Stiles B, Wang Y, Stahl A, Bassilian S, Lee WP, Kim YJ, et al. Liver-specific deletion of negative regulator Pten results in fatty liver and insulin hypersensitivity [corrected]. *Proc Natl Acad Sci U S A.* 2004 Feb 17;101(7):2082-7.
99. Mullany LK, Nelsen CJ, Hanse EA, Goggin MM, Anttila CK, Peterson M, et al. Akt-mediated liver growth promotes induction of cyclin E through a novel translational mechanism and a p21-mediated cell cycle arrest. *J Biol Chem.* 2007 Jul 20;282(29):21244-52.
100. Jackson LN, Larson SD, Silva SR, Rychahou PG, Chen LA, Qiu S, et al. PI3K/Akt activation is critical for early hepatic regeneration after partial hepatectomy. *Am J Physiol Gastrointest Liver Physiol.* 2008 Jun;294(6):G1401-10.