

Liver Regeneration and the Atrophy-Hypertrophy Complex

Robin D. Kim, M.D.,¹ Jae Sung Kim, Ph.D.,¹ Go Watanabe, M.D., Ph.D.,¹
Dagmara Mohuczy, Ph.D.,¹ and Kevin E. Behrns, M.D.¹

ABSTRACT

The atrophy-hypertrophy complex (AHC) refers to the controlled restoration of liver parenchyma following hepatocyte loss. Different types of injury (e.g., toxins, ischemia/reperfusion, biliary obstruction, and resection) elicit the same hypertrophic response in the remnant liver. The AHC involves complex anatomical, histological, cellular, and molecular processes. The signals responsible for these processes are both intrinsic and extrinsic to the liver and involve both physical and molecular events. In patients in whom resection of large liver malignancies would result in an inadequate functional liver remnant, preoperative portal vein embolization may increase the remnant liver sufficiently to permit aggressive resections. Through continued basic science research, the cellular mechanisms of the AHC may be maximized to permit curative resections in patients with potentially prohibitive liver function.

KEYWORDS: Portal vein embolization, atrophy-hypertrophy complex, liver regeneration

Objectives: Upon completion of this article, the reader should understand the cellular and molecular mechanisms of the atrophy-hypertrophy complex in the liver following both liver injury and portal vein embolization.

Accreditation: Tufts University School of Medicine (TUSM) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

Credit: TUSM designates this educational activity for a maximum of 1 *AMA PRA Category 1 Credit*TM. Physicians should only claim credit commensurate with the extent of their participation in the activity.

THE ATROPHY-HYPERTROPHY COMPLEX IN THE LIVER

The atrophy-hypertrophy complex (AHC) is the liver's regenerative response following hepatocyte loss and a dramatic demonstration of homeostasis in adult physiology. Although atrophy can be due to different types of injury (e.g., toxins, ischemia, biliary obstruction, and partial hepatectomy), the regenerative response is con-

stant as long as a minimum amount of functional liver remnant exists.

The AHC may occur by liver disease that induces atrophy by impeding bile flow, portal venous inflow, or hepatic venous outflow to a portion of the liver. This atrophy, similar to parenchymal loss from resection, induces restorative hyperplasia through liver regeneration. The AHC is defined histologically by a decrease in

¹Department of Surgery, Division of General and GI Surgery, University of Florida, Gainesville, Florida.

Address for correspondence and reprint requests: Kevin E. Behrns, M.D., Professor and Vice Chairman, Department of Surgery, Division of General and GI Surgery, University of Florida, 1600 SW Archer Road, PO Box 100286, Gainesville, FL 32610 (e-mail: Kevin.Behrns@surgery.ufl.edu).

Portal Vein Embolization; Guest Editors, David C. Madoff, M.D., and Thierry de Baere, M.D.

Semin Intervent Radiol 2008;25:92-103. Copyright © 2008 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662.
DOI 10.1055/s-2008-1076679. ISSN 0739-9529.

the hepatocyte fraction and an increase in the composition of biliary components.¹ The recognition that the AHC could be induced by portal vein embolization (PVE) to grow the potential remnant liver has allowed more aggressive liver resections while minimizing liver insufficiency. Further, the increasing use of PVE has driven research to define the mechanisms of the AHC.

In this article, the cellular and molecular mechanisms of the AHC are discussed. The roles of apoptosis and necrosis in hepatocellular atrophy and liver regeneration in compensatory hypertrophy are described, especially as they relate to PVE. Differences in these mechanisms in the healthy and diseased liver are also examined. Finally, strategies to potentiate these restorative mechanisms and their therapeutic potential are outlined.

THE AHC IN LIVER DISEASE

Biliary Obstruction

The AHC following biliary obstruction occurs when unilateral biliary obstruction induces atrophy of the ipsilateral liver and the contralateral liver undergoes hypertrophy. The AHC in this setting not only possesses the three characteristics of AHC just mentioned, but additional findings include (1) ductal inflammation and injury, (2) periductal venous injury, (3) ductal proliferation, (4) sinusoidal widening, and (5) a late progressive septal fibrosis and nodular changes.^{2,3} These findings and animal studies of bile duct ligation suggest that bile duct obstruction alone is insufficient to cause the AHC.^{4,5}

Various causes of biliary obstruction are associated with the AHC. Some studies have found the AHC in association with 18% of hilar cholangiocarcinomas,⁶ 15% of postcholecystectomy biliary strictures,⁷ 15% of primary sclerosing cholangitides,⁸ hepatolithiasis in conditions such as recurrent pyogenic cholangitis,⁹ benign bile duct tumors (e.g., papillomas, cystadenomas, and granular cell tumors), and infestations of the biliary tree (e.g., *Clonorchis sinensis* and *Ascaris lumbricoides*).¹⁰ In other space-occupying diseases such as large hepatomas, metastases, and cystic lesions (e.g., simple and hydatid cysts),^{11,12} combined biliary and portal venous obstruction may induce the AHC.

Portal Vein Obstruction

The AHC following portal vein occlusion occurs when a disease process blocks inflow through a lobar or segmental portal vein, causing atrophy of the ischemic parenchyma and hypertrophy of the uninvolved liver. Portal vein obstruction is thought to be the most important factor in AHC associated with malignancy.¹³ PVE achieves the AHC through the same mechanisms

and hence shares the histological characteristics and mechanisms that are discussed later.

Following early reports of the AHC in association with portal vein obstruction,¹⁴ various liver diseases have been implicated as underlying causes. In one series of 28 AHC cases from malignancy-induced portal vein occlusion, the tumor types were hilar cholangiocarcinoma in 21 (75%), hepatocellular carcinoma in 3 (10.7%), colon cancer metastases in 1 (3.6%), and pancreatic cancer in 1 (3.6%).⁵ Other causes of AHC from portal vein occlusion include hydatid cysts near the hilum,^{11,12} hepatolithiasis,^{11,15,16} and portal vein occlusion from hypercoagulable states resulting in cavernous transformation of the portal vein.¹⁷

Hepatic Vein Obstruction

The AHC may occur in hepatic vein obstruction. It is classically described in Budd-Chiari syndrome (BCS) in which the drainage from at least two of three hepatic veins is occluded, usually from post-thrombotic hepatic vein stenoses.¹⁸ Most cases of BCS are associated with hypercoagulable states such as primary myeloproliferative disorders, factor V Leiden mutations, anticardiolipin antibodies, and Behçet's disease. Compression of hepatic vein outflow from liver lesions/abscesses is the cause in only 5% of cases.¹⁹ The hepatic venous outflow obstruction in BCS causes increased sinusoidal pressure, sinusoidal portal hypertension, ischemic necrosis in a centrilobular distribution, and later fibrosis.¹⁸ Because the caudate lobe drains directly into the inferior vena cava, hypertrophy occurs here in 80% of patients with a chronic presentation.²⁰

THE AHC IN PORTAL VEIN EMBOLIZATION: EARLY DISCOVERIES AND CURRENT USE

In 1920, Rous and Larimore first reported that ligation of a segmental portal vein in rabbits led to atrophy of that segment and hypertrophy of the remaining liver.²¹ Later clinical studies confirmed that portal vein occlusion by tumor or ligation induced ipsilateral atrophy and contralateral hypertrophy.¹⁴ In 1990, Makuuchi et al reported the use of preoperative PVE in patients undergoing extended right-sided hepatectomy for hilar cholangiocarcinoma.²² Many studies have since confirmed the utility of PVE to allow safe extended resections.

PORTAL VEIN EMBOLIZATION, LIVER ISCHEMIA, AND ATROPHY

Occlusion of the portal vein produces hepatic ischemia. Because the liver is a highly oxygen-dependent organ, impaired blood supply rapidly causes hepatic hypoxia, which subsequently progresses to anoxia, especially in

pericentral regions of the liver lobule.^{23,24} Cardinal features of tissue ischemia are depletion of energy, loss of energy substrate, and acidosis.²⁵ Contrary to conventional belief, cells often withstand extended ischemia and maintain their functionality and viability after prolonged periods of ischemia. One mechanism allowing hepatocyte survival during ischemia is acidosis that results from hydrolysis of high-energy phosphates, accumulation of lactate, and the release of protons from acidic organelles.^{26–28} Reperfusion of tissues recovers oxygen supply and normal physiological pH. However, paradoxically, reperfusion aggravates and precipitates cell and tissue death.²⁵ The mechanisms underlying lethal ischemia/reperfusion (I/R) injury are multifactorial, including generation of reactive oxygen species (ROS), elevation of calcium, activation of injurious catabolic enzymes, and mitochondrial dysfunction.²⁹ However, restoration of pH upon reperfusion is a major and independent event causing cell death^{25,27,28,30} because reoxygenation at acidic pH prevents I/R injury, whereas recovery to normal pH without reoxygenation does not block cell death after ischemia. In this paradoxical cell death after reperfusion, onset of the mitochondrial permeability transition (MPT), a phenomenon by which the mitochondria lose the integrity of mitochondrial inner membranes, is a major causative event in both necrosis and apoptosis after I/R.^{25,27,31}

Mitochondrial Permeability Transition

The MPT, first characterized by Hunter et al in the mid-1970s,³² is a pathological phenomenon initiated by the opening of high conductance pores in the mitochondrial inner membrane.²⁵ Under normal conditions, the mitochondrial membrane is virtually impermeable to all solutes except for those having specific transporters. However, when cells are exposed to toxic insults such as I/R, oxidative stress, and hepatocellular toxins, permeability transition pores in the mitochondria open. As a consequence, all solutes with a molecular mass of up to 1500 Da can nonselectively diffuse into the mitochondria.³³ Onset of the MPT causes mitochondrial depolarization, uncoupling of oxidative phosphorylation, and large amplitude swelling leading to adenosine triphosphate (ATP) depletion and cell death.

Many agents are known to promote or prevent onset of the MPT.³⁴ Calcium loading, inorganic phosphate, alkaline pH, ROS, and nitrogen radicals promote the MPT, whereas cyclosporin A, magnesium, acidic pH, and phospholipase inhibitors all prevent opening of permeability transition pores. Inhibition of the MPT by cyclosporin A or its derivatives prevents I/R injury to hepatocytes,²⁷ myocytes,³⁵ and other cells.^{36,37} The MPT can be directly assessed in live cells using confocal microscopy with calcein, a green fluorescing fluorophore.^{27,35,38}

Hepatocellular Necrosis after Ischemia/Reperfusion (Fig. 1)

In hepatocytes, the formation of plasma membrane blebs is an initial indication of ischemic injury. This protrusion structure in the plasma membranes results from cytoskeletal alteration due to ATP depletion. Although the projection of surface blebs into the sinusoidal lumen can impair microcirculation, bleb formation is often reversible. However, rupture of blebs causes irreversible cell injury.^{39,40} After reperfusion and prior to cell death, hepatocytes develop a metastable state characterized by mitochondrial permeabilization, loss of lysosomal membrane integrity, coalescence and growth of surface blebs, and cell swelling. After rupture of the plasma membranes, cells release cytosolic enzymes and cofactors that are indispensable for cell survival. Furthermore, loss of permeability barrier in the plasma membranes causes disruption of ion homeostasis and electrical gradient. Necrosis can be assessed by cellular uptake of trypan blue or propidium iodide, which are normally excluded by healthy cells.

Hepatocellular Apoptosis after Ischemia/Reperfusion

Reperfusion of ischemic livers can cause apoptosis, characterized by cell shrinkage, caspase activation, chromatin condensation, and nuclear fragmentation.^{41,42}

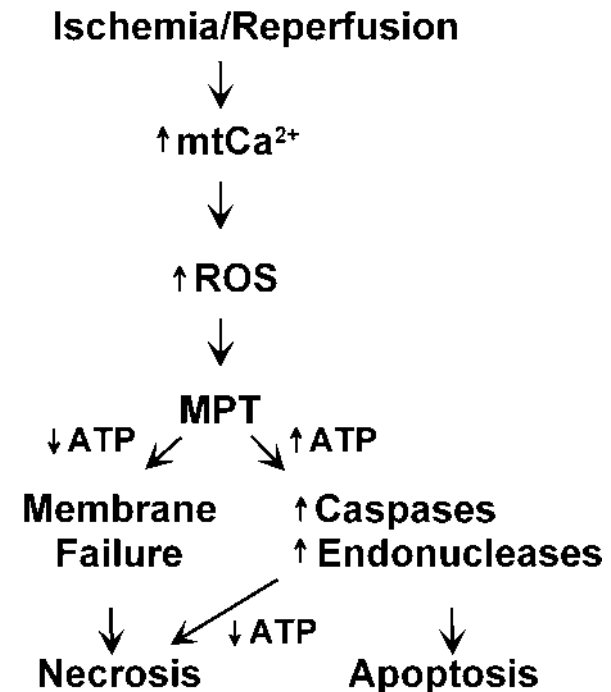


Figure 1 Events leading to hepatocyte death following ischemia/reperfusion. ATP, adenosine triphosphate; MPT, mitochondrial permeability transition; $mtCa^{2+}$, mitochondrial calcium; ROS, reactive oxygen species.

In contrast to necrosis, apoptosis develops with inflammation, scarring, and release of intracellular contents. Moreover, ATP (or deoxyadenosine triphosphate [dATP]) is required for execution of apoptotic cell death.⁴³⁻⁴⁵

As in necrosis, the mitochondria play an essential role in the development of apoptosis. A variety of proapoptotic proteins are localized in the mitochondria, including cytochrome *c*, apoptosis-inducing factor, and Smac-Diablo.⁴⁶ Release of these proteins to the cytosol initiates apoptosis, which is tightly regulated under normal circumstances. However, pathological conditions, such as I/R or overproduction of tumor necrosis factor alpha (TNF α) and Fas ligand, induce apoptosis by either a mitochondrial or non-mitochondrial pathway.^{47,48} In the mitochondrial pathway, release of cytochrome *c* from the mitochondrial intermembrane space to the cytosol forms a complex with apoptosis protease activating factor-1 (APAF-1) and ATP (or dATP), leading to activation of caspases 9 and 3.⁴⁹ The mechanisms of release of proapoptotic proteins remain controversial. One proposed mechanism is the formation of specific mitochondrial channels with the Bcl-2 family.⁵⁰ Another mechanism is that the MPT induces mitochondrial swelling, rupture of mitochondrial outer membranes, and release of cytochrome *c*.⁵¹

Necrosis or Apoptosis after Ischemia/Reperfusion?

Although necrosis is the predominant cell death pathway after I/R, apoptotic cell death often coexists with necrosis.^{52,53} Thus this question arises: "How can I/R cause two different types of cell death?" The extreme conclusions that I/R induces all necrosis or all apoptosis may be missing the important fact that both types of cell death can occur simultaneously. Indeed, pathways to necrosis and apoptosis can be shared, and onset of the MPT is a common mechanism initiating both necrosis and apoptosis after I/R and toxic stress.^{31,54} If the MPT is widespread and glycolytic energy substrate is unavailable, cells become profoundly depleted of ATP. Because ATP is an essential player in the initiation of apoptosis, ATP depletion leads to failure of the plasma membrane integrity barrier and apoptosis. However, if the MPT is limited to a small population of the mitochondria and cells maintain 15 to 20% of normal ATP, necrosis does not occur. Instead, cells develop ATP-dependent apoptosis. After ATP-consuming apoptosis continues, cells become depleted of ATP, which generates a pattern of secondary necrosis that is often observed in pathological conditions. Thus necrosis and apoptosis can be switched, and these apparent independent types of cell death are not distinct entities.

PORTAL VEIN EMBOLIZATION AND LIVER HYPERTROPHY

Anatomical and Histological Changes in the Hypertrophic Response

The liver undergoes both anatomical and histological changes from the AHC. The extent to which these changes occur vary following PVE/occlusion versus partial hepatectomy. Anatomically, the liver rotates about the hilar axis and toward the atrophic side. This rotation can change the relative locations of the bile duct (posterior), hepatic artery (anterolateral), and portal vein (anteromedial) within the hepatoduodenal ligament.⁵⁵

The rate of cell proliferation is not uniform throughout the lobule. Higher rates of cellular proliferation occur in the periportal regions (zone 1) with a gradual decrease toward the central veins (zone 3),⁵⁶ and they may reflect the relative concentration of portal venous growth factors from across the liver lobule.^{4,56} The concept of hepatotrophic factors carried in portal venous blood is further corroborated by the occurrence of atrophy that occurs when portal blood flow is absent⁴ and circadian variations in DNA synthesis related to postprandial increases in portal venous blood flow.^{57,58}

Liver Regeneration: Compensatory Hypertrophy and Hyperplasia in Response to Injury

Although only 0.0012 to 0.01% of hepatocytes in the uninjured adult liver replicate at any given time, this percentage can increase such that the volume from a two-thirds hepatectomy can be restored within weeks.^{56,59} Although loosely termed *hypertrophy*, the restorative process involves mainly hyperplasia or an increase in cell number. In addition, hypertrophy, or increased cell size, also occurs during regeneration.⁶⁰⁻⁶²

PROLIFERATIVE SIGNALING IN THE LIVER FOLLOWING PVE (FIG. 2)

The volume enlargement of the nonembolized liver lobe comes from both hepatocyte proliferation and hepatocyte hypertrophy.⁶³ Although little is known about the connection between hepatocyte hypertrophy and hepatocyte proliferation after PVE, the initiation factors that cause hepatocyte proliferation have been well investigated using hepatectomized animal models. The proliferation of the nonoccluded liver appears to be independent of the loss of liver mass because compensatory hyperplasia is initiated before atrophy of the occluded liver ensues.⁶⁴

Hemodynamic changes within the portal vein may be one signal that initiates liver regeneration. Increased portal vein pressure (shear stress) causes endothelial cells and/or hepatocytes to produce nitric oxide (NO) within 4 to 6 hours after partial hepatectomy.

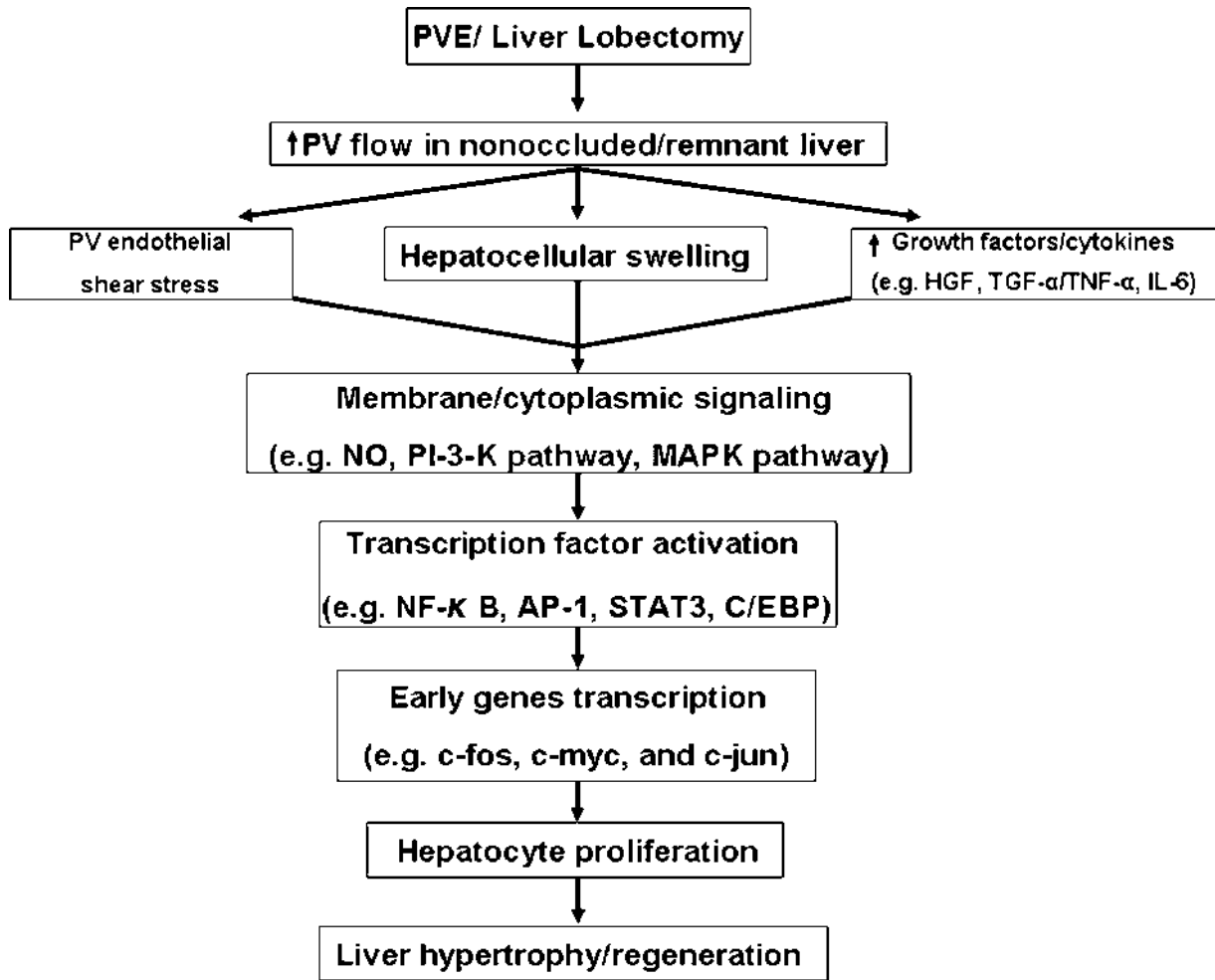


Figure 2 Changes promoting hypertrophy/regeneration in the nonoccluded/remnant liver following portal vein embolization (PVE)/liver lobectomy. HGF, hepatocyte growth factor; IL-6, interleukin-6; NO, nitric oxide; TGF α , transforming growth factor alpha; TNF α , tumor necrosis factor alpha.

Inhibition studies have shown that NO promotes DNA synthesis in hepatocytes.⁶⁵ In addition, inducible nitric oxide synthase (iNOS) knockout mice showed impaired liver regeneration and hepatocyte apoptosis.⁶⁶ Although in vitro studies have shown that NO mediates the inactivation of methionine adenosyltransferase (MAT I/III) and consequently extracellular signal-regulated kinase (ERK 1 and 2) activation,⁶⁷ these findings need to be confirmed in vivo.

Hepatocyte swelling caused by increasing portal venous flow in the nonoccluded liver lobe may be another mechanism by which liver regeneration following hepatectomy or PVE is initiated. Hepatocyte swelling can activate intracellular signaling pathway molecules such as mitogen-activated protein kinase (MAPKs), jun N-terminal kinases (JNK), and ERK.⁶⁰

Systemic circulation of growth factors may also be important in hepatocyte proliferation in the nonoccluded liver lobe as well as liver regeneration after hepatectomy. In the regenerating liver, several growth factors and cytokines are important stimuli for

hepatocyte replication and modulate subsequent transcription factors during liver regeneration. In contrast to liver regeneration after hepatectomy, little is known about the contribution of growth factors, cytokines, or transcription factors after PVE.

Growth Factors (Fig. 3) Hepatocyte growth factor (HGF) is a potent mitogen that binds the HGF receptor, c-met, and can induce hepatocyte DNA synthesis both in vitro and in vivo. HGF is produced by non-parenchymal cells in the liver and acts as a paracrine factor on hepatocytes. Uemura et al analyzed the expression of HGF mRNA in animals and showed an increase in the nonligated lobes between 6 and 24 hours, followed by a significant increase in DNA synthesis.⁶⁸ In contrast, no increase of DNA synthesis was observed in the ligated lobe, although there was a slight elevation in HGF mRNA expression. Elevated serum levels of HGF may be also important because Kaido et al reported that the liver lobes of rats with continuous HGF infusion after portal vein ligation showed increased liver weight

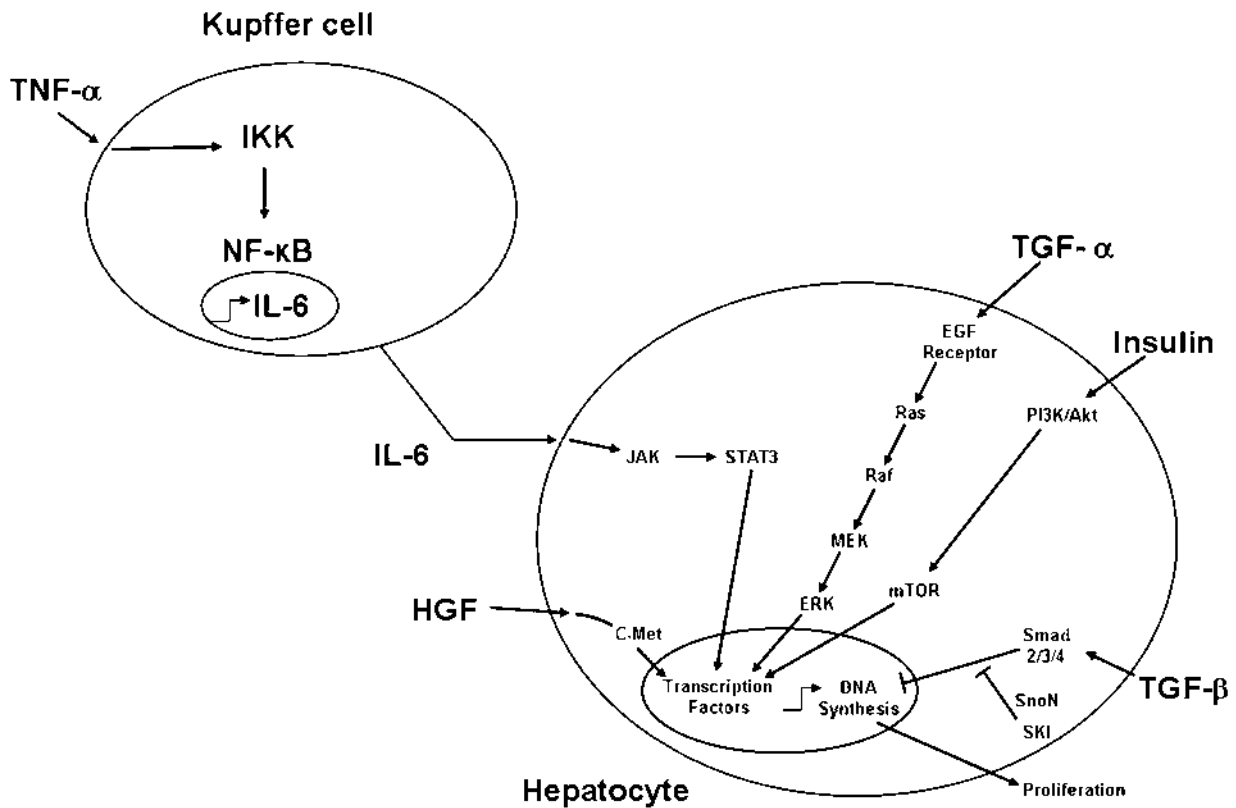


Figure 3 Signaling pathways that modulate the atrophy-hypertrophy complex. HGF, hepatocyte growth factor; IL-6, interleukin-6; TGF α , transforming growth factor alpha; TGF β , transforming growth factor beta; TNF α , tumor necrosis factor alpha.

and DNA synthesis compared with that of untreated rats.⁶⁹

Transforming growth factor alpha (TGF α), an autocrine factor produced by hepatocytes, binds the EGF receptor (EGFR) to stimulate hepatocyte replication in vitro and is important for liver regeneration after partial hepatectomy. TGF α expression in hepatocytes in both the embolized and nonembolized parts of the human liver was found to be increased.⁷⁰

The HGF receptor c-met and EGFR are members of the receptor tyrosine kinase family and induce activation of intracellular signaling pathways. Two of these pathways, the phosphoinositide-3 kinase (PI3K)-Akt-mTOR and the Ras-Raf-MEK cascades, activate several transcription factors such as CCAAT/enhancer binding protein (C/EBP) and c-jun to induce hepatocyte proliferation during liver regeneration.

Activin is a growth and differentiation factor of the transforming growth factor beta (TGF β) family that transduces signals to Smads (a class of signaling effectors described initially from *Caenorhabditis elegans* Sma and *Drosophila* Mad). Activin A, a dimeric protein of two β_A subunits, has been most extensively examined and is expressed primarily in hepatocytes.⁷¹ Activin A is a negative regulator that promotes the termination of liver regeneration. Animal studies show that the pattern of β_A

mRNA expression of the ligated and nonligated lobes are similar; the levels increase initially at 12 hours after portal vein ligation (PVL), return to baseline, and then increase maximally at 120 hours.⁷²

Cytokines (Fig. 3) TNF α is a critical cytokine for the priming of hepatocyte replication. TNF α is produced primarily by Kupffer cells during liver regeneration and activates nuclear factor-kappa B (NF- κ B) in nonparenchymal cells in an autocrine and paracrine fashion with resultant interleukin-6 (IL-6) production. Liver regeneration was shown to be inhibited by anti-TNF α antibody⁷³ and in TNF α receptor type I knockout mice.⁷⁴ TNF α may also play an important role even in the PVL model. Yokoyama et al measured the TNF α mRNA expression in the nonligated lobe by cDNA expression array and showed a fourfold increase over controls.⁷⁵ In contrast, some investigators have questioned the importance of TNF α in liver regeneration. For example, Hayashi et al observed normal liver regeneration after partial hepatectomy in TNF α knockout mice.⁷⁶

IL-6 is an important activator of signal transducers and activators of transcription (STAT3) and is released by hepatic stellate and Kupffer cells upon stimulation by TNF α . Activated STAT3 enters the nucleus and induces the transcription of specific

immediate-early genes essential for liver regeneration. IL-6 mRNA was significantly induced in both ligated and nonligated liver lobes in the first hour after portal vein ligation, but no significant difference was observed between them.⁷⁷ Others also observed similar results of IL-6 expression.⁷⁵ Kobayashi et al reported that the serum IL-6 rose and reached maximum at 6 hours after PVL and then decreased. An *in situ* hybridization study showed that IL-6 mRNA in the nonligated lobe was predominantly expressed in sinusoidal endothelial cells around the periportal area (zone 1) 3 hours after PVL.⁷⁸

Transcription Factors (Fig. 3) Stärkel et al reported changes in expression of the transcription factors NF- κ B, STAT3, c-fos, c-myc, and c-jun after portal branch ligation.⁷⁷ The increased mRNA expressions of c-fos, c-myc, and c-jun during the first 2 hours were observed in both the ligated and nonligated lobe. However, the authors cautioned that such findings should be interpreted with caution because STAT3 levels also increased with sham operations. Recently, a nonstress-PVL rat model that minimizes the effects of surgical stress was established⁷⁸ and has been used to examine the regenerative signals after PVL.⁷⁵ The patterns of NF- κ B p65, phosphorylated STAT3, c-fos, and c-jun levels differed. Nonligated lobes demonstrated biphasic activation of these transcription factors with peaks at 1 and 3 to 6 hours after PVL. However, ligated lobes showed a lower level of activation that reached maximum concentrations 2 hours after PVL.

Growth Arrest following the Hypertrophic Response (Fig. 3)

TGF β plays an important growth regulatory role in epithelial cells. TGF β binds to the type II TGF β serine/threonine kinase receptor. Then, activated type II receptor forms a dimer with the type I receptor.⁷⁹ These events activate the Smad pathway with resultant growth arrest and/or apoptosis. Through adaptors, Smads 2 and 3 are recruited to the TGF β receptor complex and phosphorylated by the type I receptor. This releases Smad 2/3 from the transmembrane complex and allows formation of a heterotrimeric complex with the common mediator, Smad 4.⁸⁰ The Smad complex then translocates to the nucleus where it activates TGF β -responsive genes through cooperative interactions with DNA and other DNA-binding proteins.

Smad activity is modulated by adaptors such as Smad anchor for receptor activation (SARA), filamin, embryonic liver Fodrin (ELF), as well as functional interactions with multiple other signal transduction pathways.⁸¹⁻⁸³ Other intracellular regulators of Smad function include microtubules that serve as cytoplasmic sequesters, controlling Smad 2 association and phosphorylation by the TGF β receptor.⁸⁴

TGF β signaling is modulated by several different mechanisms. Smad 7, a target gene of TGF β , acts at the level of the membrane receptor to inhibit Smad 2 and Smad 3 activation. Smad ubiquitin regulatory factor 1 (Smurf1) and Smurf2 associate with the nuclear Smad7 after stimulation by TGF β . The Smurfs regulate ubiquitination of ELF, which can become displaced from the signaling pathway and degraded by proteasomes.

In the nucleus, gene transactivation can be blocked by binding of the Smad complex to nuclear corepressors such as Ski, SnoN, or TGIF. Additionally, p53 modulates TGF β signaling through independent binding of the Smads.

Interestingly, even though TGF β mRNA is increased in the regenerating liver after partial hepatectomy, the proliferating hepatocytes are resistant to its growth inhibitory effects. A 2002 study provided indirect evidence of active TGF β signaling by observing an increase in Smad 2 phosphorylation during the first 5 days after hepatectomy.⁸⁵ However, concomitant increases in protein levels of SnoN (2 to 48 hours) and Ski (24 to 72 hours) and increased activity of TGF β repressors also occurred. A complex was formed between SnoN, Ski, phosphorylated-Smad 2, Smad 3, and Smad 4 during the 5 days following partial hepatectomy, thus explaining the resistance to TGF β growth arrest. TGF β signaling causes early ubiquitination and proteasome-mediated degradation of both Ski and SnoN to allow Smad-induced gene activation. The upregulation of SnoN mRNA can serve as negative feedback to return TGF β signaling to basal levels.^{86,87} Further studies are needed to clarify what controls the upregulation of Ski and SnoN in the regenerating liver and whether their degradation is blocked to promote hepatocyte proliferation.

Inhibition of growth may also involve the cyclin-dependent kinase (CDK)-inhibitory proteins (CDKIs). The p21-Cdk interacting protein 1 (p21-Cip1) has bimodal upregulation as an immediate-early gene within 30 minutes of partial hepatectomy (PH) and also from 48 to 72 hours after PH. CDKIs bind cyclin/CDK complexes and p21 to regulate CDK1, CDK2, CDK4, and CDK6. The early expression of p21-Cip1 may synchronize entry into the G1 phase. The latter expression of p21-Cip1 may limit growth. Importantly, p21-Cip1 expression is also modulated by the growth inhibitory cytokines, TGF β , and activin.⁸⁸

Hypertrophy in the Injured Liver

Patients with compromised liver function may benefit from preoperative PVE to increase the size of the future liver remnant before extended liver resections. In one prospective clinical trial assessing the efficacy of PVE, patients with or without chronic liver disease in whom an elective right hepatectomy was indicated were

assigned to have surgery either with or without PVE. The postoperative courses of those with normal livers were similar in embolized and nonembolized patients. In contrast, preoperative PVE in patients with chronic liver disease significantly decreased the incidence of postoperative complications. The authors concluded that preoperative PVE was beneficial only in patients with chronic liver disease.⁸⁹

Investigators have shown that the injured liver regenerates less efficiently following both PVE and hepatectomy. The regeneration rate of the nonembolized lobes in cirrhosis (9.3 cm³/day) was slower than that of the normal liver (11.8 cm³/day).⁹⁰ Despite this difference, others have found only an insignificant difference in the final volume of the nonembolized lobe between cirrhotic versus normal livers,⁸⁹ between livers with cirrhosis versus those with mild or moderate fibrosis,⁹¹ and between normal and injured livers due to viral hepatitis.⁹²

In addition to the histological changes in regeneration, the changes in metabolic function are also important. Hypertrophy of the nonembolized lobe was impaired in patients with high serum bilirubin at PVE.⁹³ Others have shown that liver regeneration in patients with hilar cholangiocarcinoma who underwent biliary decompression before PVE was not impaired with a growth rate of 12 cm³/day.⁹⁴ Other clinical factors found to impact liver hypertrophy negatively include diabetes mellitus^{93,94} and male gender.⁹³ The mechanisms underlying these associations remain unclear.

Some have shown that both diseased and normal livers are capable of hypertrophy following PVL. In a rat model, Lee et al induced cirrhosis with carbon tetrachloride and investigated the regenerative response following PVL.⁹⁰ Both cirrhotic and noncirrhotic hepatocytes had comparable elevations in mitotic index at 3 days, and although DNA synthesis was increased in both, it was delayed in the cirrhotic livers.

Mizuno et al studied whether cholestasis could affect the hypertrophy of the nonligated lobe after PVL. The common bile duct was ligated 5 days before PVL in a rat model, and then DNA polymerase α expression, a marker of hepatocyte replication, was measured. These studies showed that the induction of liver hypertrophy in the cholestatic rats was similar to that of the noncholestatic rats following PVL.⁹⁵

The Roles of Nonhepatocytes during Liver Regeneration

After PH, mature hepatocytes proliferate maximally at 24 hours followed by the replication of biliary ductal cells, Kupffer cells, stellate cells, and, lastly, endothelial cells.⁹⁶ One important component of the regenerative process is extracellular matrix (ECM). Urokinase-like plasminogen activator (uPA) not only converts plasmi-

nogen to plasmin but also helps in the remodeling of the ECM to allow cell division, activation of extracellular pro-metalloproteinases, and release of the bound single-chain form of HGF from the ECM.⁹⁷

The uPA also initiates the degradation of the ECM through activation of the matrix metalloproteinase (MMP) cascade.^{98,99} Within 5 minutes of PH, increased uPA activity correlates with conversion of inactive pro-MMP-2 and pro-MMP-9 to their active forms,¹⁰⁰ and it initiates disruption of the ECM.¹⁰¹ This uPA-dependent degradation of the ECM causes release of bound HGF with a subsequent increase in serum HGF concentration.^{102,103} In uPA -/- mice treated with monoclonal anti-Fas antibody (a stimulator of apoptosis), serum HGF concentrations increased later than in controls, but delayed HGF release was reversed upon transfection with the uPA gene.¹⁰⁴ Collectively, these studies suggest that uPA is an important initiator of free HGF and that ECM remodeling is needed in the early phase of liver regeneration.

"Oval cells," a normally dormant hepatic stem cell population, may also contribute to liver regeneration. These small epithelial cells possess oval nuclei, scant cytoplasm, and reside in the bile ductules and canals of Hering. These cells can differentiate into cholangiocytes or hepatocytes. Hepatocyte differentiation leads to the formation of intermediate hepatocyte-like cells, which are defined as polygonal cells with a size between that of oval cells and hepatocytes. The current view is that these cells possess the potential to regenerate the hepatic mass when mature hepatocytes are unable to do so.^{105,106} Some have demonstrated that repopulation of the liver occurs following drug-induced injury that normally precludes mature hepatocyte replication, suggesting regeneration through the stem/oval cell compartment.¹⁰⁷⁻¹⁰⁹

In addition, bone marrow or hematopoietic derived stem cells enter the liver through the portal vasculature. During and after episodes of severe liver injury, a large proportion of mature hepatocytes and cholangiocytes are derived from hematopoietic stem cells.¹¹⁰ Even lethally damaged livers regenerate after transplantation of bone marrow-derived stem cells,¹¹¹ although this process occurs through stem cells that fuse with hepatocytes rather than through pure stem cell repopulation of the liver.^{112,113} Further studies are needed to define the potential uses of stem cell transplantation as a therapy for end-stage liver disease.

STRATEGIES TO IMPROVE THE HYPERTROPHIC RESPONSE

Soon after the benefit of preoperative PVE was realized, potential strategies to maximize the hypertrophic response were evaluated. *Bone morphogenetic protein-1* (BMP-7), a protein involved in liver organogenesis,

enhanced liver regeneration following partial hepatectomy in an animal model.¹¹⁴ *Prostaglandin E(1) in lipid microspheres* (Lipo PGE[1]) significantly increases DNA synthesis and survival following 90% hepatectomy in rats.¹¹⁵ *Hepatopoietin* is a hepatotrophic growth factor that stimulates proliferation in cultured hepatocytes and hepatoma cells and liver regeneration in animal studies.¹¹⁶ *Proteases* may help control the initiation and termination of liver regeneration by releasing growth factors anchored to the extracellular matrix.¹¹⁷ *Ischemic preconditioning* has beneficial effects on liver regeneration in animal models by upregulating growth-promoting factors, suppressing growth-inhibitory factors, and preserving energy levels for regeneration.^{118,119} Finally, in one prospective study of patients undergoing preoperative PVE for the resection of liver malignancies, 6 of 13 patients received CD133 + bone marrow stem cells directly to future remnant liver segments. Those patients receiving preoperative stem cells showed significantly increased remnant liver growth as compared with controls.¹²⁰ Others have found that transplanted bone marrow cells can generate hepatocytes and help in liver repair and regeneration.¹²¹ Although preliminary, these strategies to promote liver regeneration may someday be used in conjunction with preoperative PVE to maximize the growth of the future liver remnant.

CONCLUSIONS

The AHC is a regulated compensatory response to liver injury that reestablishes adequate liver function for survival. The AHC involves complex anatomical, histological, cellular, and molecular processes that result in partial liver loss and regeneration. The signals responsible for these events are broad. They are both intrinsic and extrinsic to the liver and involve both physical forces and biochemical interactions. Differences in these mechanisms in the healthy and diseased liver may be used to maximize the liver's ability to heal. When used in conjunction with portal vein embolization, these mechanisms may someday be exploited to permit curative resections in patients with marginal liver function.

ACKNOWLEDGMENTS

We would like to thank Jason E. Cline for his assistance in formatting this article.

REFERENCES

- Bellentani S, Hardison WG, Manenti F. Mechanisms of liver adaptation to prolonged selective biliary obstruction (SBO) in the rat. *J Hepatol* 1985;1:525-535
- Schaffner F, Bacchin PG, Hutterer F, et al. Mechanism of cholestasis: 4. Structural and biochemical changes in the liver and serum in rats after bile duct ligation. *Gastroenterology* 1971;60:888-897
- Gall JA, Bhathal PS. Origin and involution of hyperplastic bile ductules following total biliary obstruction. *Liver* 1990;10:106-115
- Schweizer W, Duda P, Tanner S, et al. Experimental atrophy/hypertrophy complex (AHC) of the liver: portal vein, but not bile duct obstruction, is the main driving force for the development of AHC in the rat. *J Hepatol* 1995;23:71-78
- Hann LE, Getrajdman GI, Brown KT, et al. Hepatic lobar atrophy: association with ipsilateral portal vein obstruction. *AJR Am J Roentgenol* 1996;167:1017-1021
- Hadjis NS, Adam A, Gibson R, Blenkharn JI, Benjamin IS, Blumgart LH. Nonoperative approach to hilar cancer determined by the atrophy-hypertrophy complex. *Am J Surg* 1989;157:395-399
- Hadjis NS, Blumgart LH. Role of liver atrophy, hepatic resection and hepatocyte hyperplasia in the development of portal hypertension in biliary disease. *Gut* 1987;28:1022-1028
- Hadjis NS, Adam A, Blenkharn I, Hatzis G, Benjamin IS, Blumgart LH. Primary sclerosing cholangitis associated with liver atrophy. *Am J Surg* 1989;158:43-47
- Jeyarajah DR. Recurrent pyogenic cholangitis. *Curr Treat Options Gastroenterol* 2004;7:91-98
- Rana SS, Bhasin DK, Nanda M, Singh K. Parasitic infestations of the biliary tract. *Curr Gastroenterol Rep* 2007;9:156-164
- Rozanes I, Acunas B, Celik L, Minareci O, Gokmen E. CT in lobar atrophy of the liver caused by alveolar echinococcosis. *J Comput Assist Tomogr* 1992;16:216-218
- Karabulut K, Ozden I, Poyanli A, et al. Hepatic atrophy-hypertrophy complex due to *Echinococcus granulosus*. *J Gastrointest Surg* 2006;10:407-412
- Lorigan JG, Charnsangavej C, Carrasco CH, Richli WR, Wallace S. Atrophy with compensatory hypertrophy of the liver in hepatic neoplasms: radiographic findings. *AJR Am J Roentgenol* 1988;150:1291-1295
- Bax HR, Mansens BJ, Schalm L. Atrophy of the liver after occlusion of the bile ducts or portal vein and compensatory hypertrophy of the unoccluded portion and its clinical importance. *Gastroenterology* 1956;31:131-155
- Ishida H, Naganuma H, Konno K, et al. Lobar atrophy of the liver. *Abdom Imaging* 1998;23:150-153
- Kusano S, Okada Y, Endo T, Yokoyama H, Ohmiya H, Atari H. Oriental cholangiohepatitis: correlation between portal vein occlusion and hepatic atrophy. *AJR Am J Roentgenol* 1992;158:1011-1014
- Vilgrain V, Condat B, Bureau C, et al. Atrophy-hypertrophy complex in patients with cavernous transformation of the portal vein: CT evaluation. *Radiology* 2006;241:149-155
- Valla DC. The diagnosis and management of the Budd-Chiari syndrome: consensus and controversies. *Hepatology* 2003;38:793-803
- Denninger MH, Chait Y, Casadevall N, et al. Cause of portal or hepatic venous thrombosis in adults: the role of multiple concurrent factors. *Hepatology* 2000;31:587-591
- Matthieu D, Kracht M, Zafrani E, Dhumeaux D, Vasile N. Budd-Chiari syndrome. In: Ferrucci J, Matthieu D, eds. *Advances in Hepatobiliary Radiology*. St. Louis: CV Mosby; 1990:3-28

21. Rous P, Larimore LD. Relation of the portal blood flow to liver maintenance: a demonstration of liver atrophy conditional on compensation. *J Exp Med* 1920;31:609–632
22. Makuuchi M, Thai BL, Takayasu K, et al. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* 1990;107:521–527
23. Lemasters JJ, Ji S, Thurman RG. Centrilobular injury following hypoxia in isolated, perfused rat liver. *Science* 1981;213(4508):661–663
24. Jungermann K, Kietzmann T. Oxygen: modulator of metabolic zonation and disease of the liver. *Hepatology* 2000;31(2):255–260
25. Kim J-S, He L, Lemasters JJ. Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. *Biochem Biophys Res Commun* 2003;304(3):463–470
26. Gores GJ, Nieminen AL, Wray BE, Herman B, Lemasters JJ. Intracellular pH during “chemical hypoxia” in cultured rat hepatocytes: protection by intracellular acidosis against the onset of cell death. *J Clin Invest* 1989;83(2):386–396
27. Qian T, Nieminen AL, Herman B, Lemasters JJ. Mitochondrial permeability transition in pH-dependent reperfusion injury to rat hepatocytes. *Am J Physiol* 1997;273: C1783–C1792
28. Currin RT, Gores GJ, Thurman RG, Lemasters JJ. Protection by acidotic pH against anoxic cell killing in perfused rat liver: evidence for a pH paradox. *FASEB J* 1991;5(2):207–210
29. McCord JM. Oxygen-derived free radicals in postschemic tissue injury. *N Engl J Med* 1985;312(3):159–163
30. Kim JS, He L, Qian T, Lemasters JJ. Role of the mitochondrial permeability transition in apoptotic and necrotic death after ischemia/reperfusion injury to hepatocytes. *Curr Mol Med* 2003;3(6):527–535
31. Kim J-S, Qian T, Lemasters JJ. Mitochondrial permeability transition in the switch from necrotic to apoptotic cell death in ischemic rat hepatocytes. *Gastroenterology* 2003;124: 494–503
32. Hunter DR, Haworth RA, Southard JH. Relationship between configuration, function, and permeability in calcium-treated mitochondria. *J Biol Chem* 1976;251(16): 5069–5077
33. Bernardi P. Mitochondrial transport of cations: channels, exchangers, and permeability transition. *Physiol Rev* 1999; 79(4):1127–1155
34. Gunter TE, Pfeiffer DR. Mechanisms by which mitochondria transport calcium. *Am J Physiol* 1990;258:C755–C786
35. Kim J-S, Jin Y, Lemasters JJ. Reactive oxygen species, but not Ca^{2+} overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia/reperfusion. *Am J Physiol Heart Circ Physiol* 2006;290(5):H2024–H2034
36. Zazueta C, Franco M, Correa F, et al. Hypothyroidism provides resistance to kidney mitochondria against the injury induced by renal ischemia-reperfusion. *Life Sci* 2007;80(14): 1252–1258
37. Li PA, Uchino H, Elmer E, Siesjo BK. Amelioration by cyclosporin A of brain damage following 5 or 10 min of ischemia in rats subjected to preischemic hyperglycemia. *Brain Res* 1997;753(1):133–140
38. Kim J-S, Ohshima S, Padiaditakis P, Lemasters JJ. Nitric oxide protects rat hepatocytes against reperfusion injury mediated by the mitochondrial permeability transition. *Hepatology* 2004;39:1533–1543
39. Herman B, Nieminen AL, Gores GJ, Lemasters JJ. Irreversible injury in anoxic hepatocytes precipitated by an abrupt increase in plasma membrane permeability. *FASEB J* 1988;2(2):146–151
40. Nieminen AL, Gores GJ, Wray BE, Tanaka Y, Herman B, Lemasters JJ. Calcium dependence of bleb formation and cell death in hepatocytes. *Cell Calcium* 1988;9(5-6):237–246
41. Gao W, Bentley RC, Madden JF, Clavien PA. Apoptosis of sinusoidal endothelial cells is a critical mechanism of preservation injury in rat liver transplantation. *Hepatology* 1998;27(6):1652–1660
42. Natori S, Selzner M, Valentino KL, et al. Apoptosis of sinusoidal endothelial cells occurs during liver preservation injury by a caspase-dependent mechanism. *Transplantation* 1999;68(1):89–96
43. Richter C, Schweizer M, Cossarizza A, Franceschi C. Control of apoptosis by the cellular ATP level. *FEBS Lett* 1996;378(2):107–110
44. Leist M, Single B, Castoldi AF, Kuhnle S, Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med* 1997;185(8):1481–1486
45. Eguchi Y, Shimizu S, Tsujimoto Y. Intracellular ATP levels determine cell death fate by apoptosis or necrosis. *Cancer Res* 1997;57(10):1835–1840
46. Galluzzi L, Larochette N, Zamzami N, Kroemer G. Mitochondria as therapeutic targets for cancer chemotherapy. *Oncogene* 2006;25(34):4812–4830
47. Scaffidi C, Fulda S, Srinivasan A, et al. Two CD95 (APO-1/Fas) signaling pathways. *EMBO J* 1998;17(6):1675–1687
48. Hatano E, Bradham CA, Stark A, Iimuro Y, Lemasters JJ, Brenner DA. The mitochondrial permeability transition augments Fas-induced apoptosis in mouse hepatocytes. *J Biol Chem* 2000;275(16):11814–11823
49. Wang X. The expanding role of mitochondria in apoptosis. *Genes Dev* 2001;15(22):2922–2933
50. Wei MC, Zong WX, Cheng EH, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science* 2001;292(5517):727–730
51. Zamzami N, Susin SA, Marchetti P, et al. Mitochondrial control of nuclear apoptosis. *J Exp Med* 1996;183(4):1533–1544
52. Gujral JS, Bucci TJ, Farhood A, Jaeschke H. Mechanism of cell death during warm hepatic ischemia-reperfusion in rats: apoptosis or necrosis? *Hepatology* 2001;33(2):397–405
53. Kohli V, Madden JF, Bentley RC, Clavien PA. Calpain mediates ischemic injury of the liver through modulation of apoptosis and necrosis. *Gastroenterology* 1999;116(1):168–178
54. Qian T, Herman B, Lemasters JJ. The mitochondrial permeability transition mediates both necrotic and apoptotic death of hepatocytes exposed to Br-A23187. *Toxicol Appl Pharmacol* 1999;154(2):117–125
55. Czerniak A, Soreide O, Gibson RN, et al. Liver atrophy complicating benign bile duct strictures: surgical and interventional radiologic approaches. *Am J Surg* 1986;152: 294–300
56. Michalopoulos GK, DeFrances MC. Liver regeneration. *Science* 1997;276:60–66

57. Barbason H, Bouzahzah B, Herens C, et al. Circadian synchronization of liver regeneration in adult rats: the role played by adrenal hormones. *Cell Tissue Kinet* 1989;22:451-460
58. Souto M, Llanos JM. The circadian optimal time for hepatectomy in the study of liver regeneration. *Chronobiol Int* 1985;2:169-175
59. Koniaris LG, McKillop IH, Schwartz SI, Zimmers TA. Liver regeneration. *J Am Coll Surg* 2003;197:634-659
60. Kim RD, Stein GS, Chari RS. Impact of cell swelling on proliferative signal transduction in the liver. *J Cell Biochem* 2001;83:56-69
61. Nagy P, Teramoto T, Factor VM, et al. Reconstitution of liver mass via cellular hypertrophy in the rat. *Hepatology* 2001;33:339-345
62. Fausto N. Liver regeneration. *J Hepatol* 2000;32:19-31
63. Komori K, Nagino M, Nimura Y. Hepatocyte morphology and kinetics after portal vein embolization. *Br J Surg* 2006;93(6):745-751
64. Lambotte L, Li B, Leclercq I, Sempoux C, Saliez A, Horsmans Y. The compensatory hyperplasia (liver regeneration) following ligation of a portal branch is initiated before the atrophy of the deprived lobes. *J Hepatol* 2000;32(6):940-945
65. Hortelano S, Dewez B, Genaro AM, Diaz-Guerra MJ, Bosca L. Nitric oxide is released in regenerating liver after partial hepatectomy. *Hepatology* 1995;21(3):776-786
66. Rai RM, Lee FY, Rosen A, et al. Impaired liver regeneration in inducible nitric oxide synthase deficient mice. *Proc Natl Acad Sci U S A* 1998;95(23):13829-13834
67. Garcia-Trevijano ER, Martinez-Chantar ML, Latasa MU, Mato JM, Avila MA. NO sensitizes rat hepatocytes to proliferation by modifying S-adenosylmethionine levels. *Gastroenterology* 2002;122(5):1355-1363
68. Uemura T, Miyazaki M, Hirai R, et al. Different expression of positive and negative regulators of hepatocyte growth in growing and shrinking hepatic lobes after portal vein branch ligation in rats. *Int J Mol Med* 2000;5(2):173-179
69. Kaido T, Yoshikawa A, Seto S, et al. Hepatocyte growth factor supply accelerates compensatory hypertrophy caused by portal branch ligation in normal and jaundiced rats. *J Surg Res* 1999;85(1):115-119
70. Kusaka K, Imamura H, Tomiya T, Takayama T, Makuuchi M. Expression of transforming growth factor- α and - β in hepatic lobes after hemihepatic portal vein embolization. *Dig Dis Sci* 2006;51(8):1404-1412
71. Vejda S, Cranfield M, Peter B, et al. Expression and dimerization of the rat activin subunits betaC and betaE: evidence for the formation of novel activin dimers. *J Mol Endocrinol* 2002;28(2):137-148
72. Takamura K, Tsuchida K, Miyake H, Tashiro S, Sugino H. Activin and activin receptor expression changes in liver regeneration in rat. *J Surg Res* 2005;126(1):3-11
73. Akerman P, Cote P, Yang SQ, et al. Antibodies to tumor necrosis factor- α inhibit liver regeneration after partial hepatectomy. *Am J Physiol* 1992;263(4):G579-G585
74. Yamada Y, Kirillova I, Peschon JJ, Fausto N. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *Proc Natl Acad Sci U S A* 1997;94(4):1441-1446
75. Yokoyama S, Yokoyama Y, Kawai T, et al. Biphasic activation of liver regeneration-associated signals in an early stage after portal vein branch ligation. *Biochem Biophys Res Commun* 2006;349(2):732-739
76. Hayashi H, Nagaki M, Imose M, et al. Normal liver regeneration and liver cell apoptosis after partial hepatectomy in tumor necrosis factor- α -deficient mice. *Liver Int* 2005;25(1):162-170
77. Stärkel P, Horsmans Y, Sempoux C, et al. After portal branch ligation in rat, nuclear factor kappaB, interleukin-6, signal transducers and activators of transcription 3, c-fos, c-myc, and c-jun are similarly induced in the ligated and nonligated lobes. *Hepatology* 1999;29(5):1463-1470
78. Kobayashi S, Nagino M, Yokoyama Y, Nimura Y, Sokabe M. Evaluation of hepatic interleukin-6 secretion following portal vein ligation using a minimal surgical stress model. *J Surg Res* 2006;135(1):27-33
79. Wrana JL, Attisano L, Wieser R, Ventura F, Massague J. Mechanism of activation of the TGF- β receptor. *Nature* 1994;370(6488):341-347
80. Abdollah S, Macias-Silva M, Tsukazaki T, et al. TbetaRI phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signaling. *J Biol Chem* 1997;272(44):27678-27685
81. Wu G, Chen YG, Ozdamar B, et al. Structural basis of Smad2 recognition by the Smad anchor for receptor activation. *Science* 2000;287(5450):92-97
82. Hococevar BA, Smine A, Xu XX, Howe PH. The adaptor molecule Disabled-2 links the transforming growth factor beta receptors to the Smad pathway. *EMBO J* 2001;20(11):2789-2801
83. Mishra L, Marshall B. Adaptor proteins and ubiquitinators in TGF- β signaling. *Cytokine Growth Factor Rev* 2006;17(1-2):75-87
84. Dong C, Li Z, Alvarez RJ, Feng XH, Goldschmidt-Clermont PJ. Microtubule binding to Smads may regulate TGF beta activity. *Mol Cell* 2000;5(1):27-34
85. Macias-Silva M, Li W, Leu JI, Crissey MA, Taub R. Up-regulated transcriptional repressors SnoN and Ski bind Smad proteins to antagonize transforming growth factor-beta signals during liver regeneration. *J Biol Chem* 2002;277(32):28483-28490
86. Stroschein SL, Wang W, Zhou S, Zhou Q, Luo K. Negative feedback regulation of TGF- β signaling by the SnoN oncoprotein. *Science* 1999;286(5440):771-774
87. Sun Y, Liu X, Ng-Eaton E, Lodish HF, Weinberg RA. SnoN and Ski protooncoproteins are rapidly degraded in response to transforming growth factor beta signaling. *Proc Natl Acad Sci U S A* 1999;96(22):12442-12447
88. Albrecht JH, Meyer AH, Hu MY. Regulation of cyclin-dependent kinase inhibitor p21(WAF1/Cip1/Sdi1) gene expression in hepatic regeneration. *Hepatology* 1997;25(3):557-563
89. Farges O, Belghiti J, Kianmanesh R, et al. Portal vein embolization before right hepatectomy: prospective clinical trial. *Ann Surg* 2003;237(2):208-217
90. Lee KC, Kinoshita H, Hirohashi K, Kubo S, Iwasa R. Extension of surgical indications for hepatocellular carcinoma by portal vein embolization. *World J Surg* 1993;17(1):109-115
91. Azoulay D, Castaing D, Krissat J, et al. Percutaneous portal vein embolization increases the feasibility and safety of major liver resection for hepatocellular carcinoma in injured liver. *Ann Surg* 2000;232(5):665-672

92. Wakabayashi H, Ishimura K, Okano K, et al. Application of preoperative portal vein embolization before major hepatic resection in patients with normal or abnormal liver parenchyma. *Surgery* 2002;131(1):26–33
93. Imamura H, Shimada R, Kubota M, et al. Preoperative portal vein embolization: an audit of 84 patients. *Hepatology* 1999;29(4):1099–1105
94. Nagino M, Nimura Y, Kamiya J, et al. Changes in hepatic lobe volume in biliary tract cancer patients after right portal vein embolization. *Hepatology* 1995;21(2):434–439
95. Mizuno S, Nimura Y, Suzuki H, Yoshida S. Portal vein branch occlusion induces cell proliferation of cholestatic rat liver. *J Surg Res* 1996;60(1):249–257
96. Michalopoulos GK. Liver regeneration. *J Cell Physiol* 2007; 213:286–300
97. Mangnall D, Smith K, Bird NC, Majeed AW. Early increases in plasminogen activator activity following partial hepatectomy in humans. *Comp Hepatol* 2004;3(1):11
98. Blasi F. Urokinase and urokinase receptor: a paracrine/autocrine system regulating cell migration and invasiveness. *Bioessays* 1993;15(2):105–111
99. Stetler-Stevenson WG. Dynamics of matrix turnover during pathologic remodeling of the extracellular matrix. *Am J Pathol* 1996;148(5):1345–1350
100. Kim TH, Mars WM, Stolz DB, Michalopoulos GK. Expression and activation of pro-MMP-2 and pro-MMP-9 during rat liver regeneration. *Hepatology* 2000;31(1):75–82
101. Mars WM, Liu ML, Kitson RP, Goldfarb RH, Gabauer MK, Michalopoulos GK. Immediate early detection of urokinase receptor after partial hepatectomy and its implications for initiation of liver regeneration. *Hepatology* 1995;21(6):1695–1701
102. Liu ML, Mars WM, Zarnegar R, Michalopoulos GK. Uptake and distribution of hepatocyte growth factor in normal and regenerating adult rat liver. *Am J Pathol* 1994; 144(1):129–140
103. Naldini L, Vigna E, Bardelli A, Follenzi A, Galimi F, Comoglio PM. Biological activation of pro-HGF (hepatocyte growth factor) by urokinase is controlled by a stoichiometric reaction. *J Biol Chem* 1995;270(2):603–611
104. Shimizu M, Hara A, Okuno M, et al. Mechanism of retarded liver regeneration in plasminogen activator-deficient mice: impaired activation of hepatocyte growth factor after Fas-mediated massive hepatic apoptosis. *Hepatology* 2001;33(3):569–576
105. Thorgeirsson SS, Everts RP, Bisgaard HC, Fujio K, Hu Z. Hepatic stem cell compartment: activation and lineage commitment. *Proc Soc Exp Biol Med* 1993;204(3):253–260
106. Oh SH, Witek RP, Bae SH, et al. Bone marrow-derived hepatic oval cells differentiate into hepatocytes in 2-acetylaminofluorene/partial hepatectomy-induced liver regeneration. *Gastroenterology* 2007;132(3):1077–1087
107. Gordon GJ, Coleman WB, Grisham JW. Temporal analysis of hepatocyte differentiation by small hepatocyte-like progenitor cells during liver regeneration in retrorsine-exposed rats. *Am J Pathol* 2000;157(3):771–786
108. Gordon GJ, Coleman WB, Hixson DC, Grisham JW. Liver regeneration in rats with retrorsine-induced hepatocellular injury proceeds through a novel cellular response. *Am J Pathol* 2000;156(2):607–619
109. Fujio K, Everts RP, Hu Z, Marsden ER, Thorgeirsson SS. Expression of stem cell factor and its receptor, c-kit, during liver regeneration from putative stem cells in adult rat. *Lab Invest* 1994;70(4):511–516
110. Roskams TA, Libbrecht L, Desmet VJ. Progenitor cells in diseased human liver. *Semin Liver Dis* 2003;23(4):385–396
111. Lagasse E, Connors H, Al-Dhalimy M, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med* 2000;6(11):1229–1234
112. Wang X, Willenbring H, Akkari Y, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* 2003;422(6934):897–901
113. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. *Nature* 2003;422(6934):901–904
114. Sugimoto H, Yang C, LeBleu VS, et al. BMP-7 functions as a novel hormone to facilitate liver regeneration. *FASEB J* 2007;21:256–264
115. Ando K, Miyazaki M, Shimizu H, Okuno A, Nakajima N. Beneficial effects of prostaglandin E(1) incorporated in lipid microspheres on liver injury and regeneration after 90% partial hepatectomy in rats. *Eur Surg Res* 2000;32:155–161
116. Gatzidou E, Kouraklis G, Theocharis S. Insights on augments of liver regeneration cloning and function. *World J Gastroenterol* 2006;12:4951–4958
117. Mohammed FF, Khokha R. Thinking outside the cell: proteases regulate hepatocyte division. *Trends Cell Biol* 2005;15:555–563
118. Gomez D, Homer-Vanniasinkam S, Graham AM, Prasad KR. Role of ischaemic preconditioning in liver regeneration following major liver resection and transplantation. *World J Gastroenterol* 2007;13:657–670
119. Jaeschke H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G15–G26
120. Fürst G, Schulte am Esch J, Poll LW, et al. Portal vein embolization and autologous CD133 + bone marrow stem cells for liver regeneration: initial experience. *Radiology* 2007;243:171–179
121. Dorrell C, Grompe M. Liver repair by intra- and extrahepatic progenitors. *Stem Cell Rev* 2005;1:61–64