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Peroxisome Proliferator-Activated Receptor gamma negatively regulates liver regeneration after partial hepatectomy via the HGF/c-met/ERK1/2 pathway

Lei Liu

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ABBREVIATIONS

15d-PGJ2	15-deoxy-(12,14)-prostaglandin J2
ALT	Alanine-aminotransferase
AMPK	Energy sensitive AMP-activated protein kinase
AP1	Activator protein 1
AST	Aspartate-aminotransferase
C/EBP	CCAAT-enhancer-binding protein
CAP	c-Cbl-associated protein
CCl_4	Carbon tetrachloride
c-Met	Hepatocyte growth factor receptor
ECM	Extracellular matrix
EGF	Epidermal growth factor like growth factor
Erk	Extracellular-signal regulated kinase
FFA	Free fatty acid
FGF	Fibroblast growth factor
FXR	Farnesoid X receptor
gDNA	Genomic DNA
GLUT	Glucose transporter
GPC3	Glypican 3
GSIS	Glucose-stimulated insulin secretion
GSK3	Glycogen synthase kinase 3
HbA1c	Glycated hemoglobin
HB-EGF	Heparin-binding epidermal growth factor like growth factor
HEstain	Hematoxylin and eosin stain
HGF	Hepatocyte growth factor
HODE	Hydroxy-octadecadienoic acids
IGFBP	Insulin like growth factor binding protein
IHC	Immunohistochemistry
IL-1	Interleukin 1
IL-6	Interleukin 6
ILK	Integrin-linked kinase
iNOS	Inducible nitric oxide synthase
JAKs	Janus kinases

LPS	Lipopolysaccharide
LTα	Lymphotoxin alpha
LTβ	Lymphotoxin beta
MAPK	Mitogen-activated protein kinases
MEK	Mitogen-activated protein kinase
MKK4	Mitogen-activated protein kinase 4
MMP-9	Matrix metallopeptidase
mTOR	Mammalian target of rapamycin
NF-κB	Nuclear factor kappa-B
NIK	NF-κB-inducing kinase
NPC	Non-parenchymal cells
OSM	Oncostatin M
PAI	Plasminogen-activator inhibitor
PCR	Polymerase chain reaction
PGC	PPAR-gamma coactivator
PH	Partial hepatectomy
PI3K	Phosphatidylinositol 3 kinase
PPAR	Peroxisome proliferator activated receptor
Rb	Retinoblastoma
RXR	Retinoid X receptor
SCF	Stem cell factor
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SOCS	Suppressors of cytokine signaling
SRC-1	Steroid receptor coactivator 1
STAT3	Signal transducer and activator of transcription 3
TGFα	Transforming growth factor
TGs	Triglyceride
TNF	Tumor necrosis factor
TSP-1	Thrombospondin-1
TZDs	Thiazolidinediones
uPA	Urokinase-type plasminogen activator
VEGF	Vascular endothelial growth factor
VSMCs	Vascular smooth muscle cells

1 INTRODUCTION

1.1 Liver regeneration

1.1.1 Physiology and central function of the liver

As the largest glandular organ of the mammalian body, the liver has a wide range of functions, including protein synthesis, detoxification and production of biochemicals. This gland plays a central role in maintaining metabolic homeostasis and serves as the primary regulatory site for energy metabolism, synthesizes essential proteins, enzymes and co-factors for digestion. Additionally, the liver also provides important immunologic functions. Due to its extensive functions, the ultrastructure of the liver consists of various cell populations of complex organization. As is currently understood, the liver is composed of both parenchymal cells (hepatocytes) and non-parenchymal cells (NPC), including resident immune cells (Kupffer cells, NK and NKT cells, T-lymphocytes), a population of mesenchymal cells (stellate cells and liver myofibroblasts) and specialized cells (biliary epithelial cells/cholangiocytes and sinusoidal endothelial cells) (*Michalopoulos GK, 2007; Michalopoulos GK, 1997*).

Hepatocytes constitute 80% of the total liver mass and exist as large, irregular polyhedral-shaped, polyploid cells arranged in thin layers that radiate from the central canal (central vein) to the periphery of the lobule (portal triad) (*Motta PM, 1977; Motta PM, 1984*). Hepatocytes carry out the major functions of the liver and are involved in protein synthesis, protein storage, producing clotting factors and serum albumin, synthesis of cholesterol, bile salts and phospholipids, transformation of carbohydrates, detoxification, modification, and excretion of exogenous and endogenous substances, and initialization of the formation and secretion of bile.

Stellate cells or Ito cells represent 5-8% of the total population of liver cells; they are located in the liver perisinusoidal space and serve as extracellular matrix (ECM) protein-producing cells but also as fat and vitamin A-storage cells. Kupffer cells, liver-specific macrophages located in the lining of the walls of the sinusoids, are essential for the phagocytosis of foreign organisms in the liver and represent the main source of cytokines and inflammatory factors (*Taub R, 2004*). Lymphocytes play an important role for innate immunity and defense against infection in the liver. Biliary epithelial cells

or cholangiocytes are cuboidal epithelial cells found in the small interlobular bile ducts, but become columnar and mucus-secreting in larger bile ducts approaching the porta hepatis and the extrahepatic ducts, and mainly contribute to bile secretion (*Tietz PS, et al., 2006*). Sinusoidal endothelial cells, composing the circulatory blood vessels that separate hepatocytes from circulating blood, play an important role in hepatic microcirculation. In addition to these common cells, the oval cells, which are considered to be facultative hepatic progenitor cells, can be found in damaged livers only (*Fausto N, et al., 2004*).

1.1.2 General features of liver regeneration

The liver has a unique capability of precisely regulating compensatory hypertrophy and hyperplasia to restore the loss of functional mass response to injury caused by surgical resection, chemicals or viral infections (*Michalopoulos GK, 1997*). Liver regeneration may be described as an orchestrated response induced by specific external stimuli and involving sequential changes in gene expression, growth factor production and morphologic structure. This regenerative capacity is important because it stipulates the clinical outcome of a serious hepatic injury, cancer resection, and living donor liver transplantation (*Nanashima A, et al., 2009*). It is notable that, unlike the majority of other regenerated mammalian organs, functional parenchymal cells generate hepatocytes to restore the original mass in the case of partial hepatectomy (PH); moreover, some chemical liver injuries induce hepatic regeneration (*Wang X, et al., 2003; Court FG, et al., 2002*). Progenitor or stem cells usually replicate and differentiate into hepatocytes in other cases of chronic chemical liver injury, including galactosamine toxicity (*Lemire JM, et al., 1991*). These features make the liver the only organ in mammals that can rapidly regain its size, structure, and function from as little as 10% of the remaining tissue (*Myronovych A, et al., 2008*). In addition, the expanded liver after regeneration does not regain its original gross anatomical structure.

Different liver injury models had been used to derive the majority of evidence defining the molecular mechanisms associated with liver regeneration. After PH, the remaining hepatocytes leave their quiescent state (G0), enter the cell cycle and proliferate, followed by the non-parenchymal cells (NPC). During this regenerative process, cells maintain their major morphologic features, have an active urea cycle, continue to metabolize drugs and synthesize albumin. In general terms, the

replicative response of quiescent hepatocytes after PH involves multiple steps, and each step is precisely controlled and regulated.

1.1.3 Models of liver injury

To simulate different causes of induced liver regeneration in patients, a number of models have been proposed for study of liver regeneration in mouse or rat. (1) A surgical model of different PH severity. The rodent model of two-thirds hepatectomy was first proposed by Higgins and Anderson (*Higgins GM, et al., 1931*), in which specific liver lobes that account for about 2/3 of the total mass are removed intact and the residual lobes regenerate the entire mass. 90% hepatectomy is also performed to induce acute liver failure. (2) A chemical-mediated hepatotoxic injury based model, in which liver injury is induced by carbon tetrachloride (CCl_4), D-galactosamine, acetaminophen, etc., has also served as a common liver injury model. The CCl_4-mediated injury based model is most widely used; it triggers necrosis of the lobular zones of the liver, which would be removed during an initial inflammatory response, followed by a regenerative response in the remaining liver cells (*Michalopoulos GK, 2007*). Notably, an intense inflammatory response is thought to be involved both at the onset and throughout of the liver regeneration response. Regarding the other chemicals, D-galactosamine causes an intracellular deficiency of uridine metabolites, leading to serious liver damage in animal models (*Decker K, et al., 1972*). Acetaminophen prevents the liver from performing the necessary breakdown steps of glucuronidation and sulfation, and the P450 system takes over, causing acute liver failure in clinical patients (*Rahman TM, et al., 2000*). Of these models, PH has several natural advantages compared with chemical-mediated hepatotoxic injury based models. It does not cause tissue damage to the residual lobes, precisely defines the initiation of the regenerative stimulus, and has high reproducibility. Considering that PH is also performed in clinical settings for solitary liver metastasis resection, trauma repair, living donor liver transplantation, etc., and due to the current lack of an adequate model to study liver regeneration in humans, PH in a porcine model becomes a model of choice in which the process of liver regeneration can be studied. (3) Genetically modified animal models. These animals may have inborn errors of metabolism and have also been proposed to serve as models of liver regeneration. In such types of animals, the liver is capable of engraftment and significant repopulation with mature human hepatocytes after xenogeneic transplantation (*Azuma H, et al., 2007*).

1.1.4 Mechanisms of liver regeneration

As mentioned above, the liver plays important roles in metabolic homeostasis. Liver regeneration is necessary to maintain normal liver function after liver resection or injury. Many studies have been performed to study the mechanism of liver regeneration. The central questions regarding the process of liver regeneration include: what are the trigger signals for the early events in the regenerative process; how are the architecture and function of liver retained during regeneration; what are the signals that turn off the growth response once the mass of the liver is reconstituted? Investigators have begun to answer these questions by using molecular and genetic approaches to identify the important regulatory pathways which control the regenerative process.

The evolution of ideas pertaining to the mechanisms of liver regeneration also changed from the original view that a single humoral agent could function as a key, capable of unlocking all of the events required for liver regeneration to the more recent idea that the activity of multiple interconnected pathways are required for liver regeneration. This idea can be demonstrated through the finding that genetic modifications resulting in defects in a single signaling pathway often result in delayed liver regeneration but do not completely block the regenerative process. Liver regeneration does require the activation of a complex network of pathways to proceed in an optimal manner. The recent literature suggests that the essential circuitry required for liver regeneration consists of three types of pathways, including various cytokines, responsible for hepatocyte priming; growth factors, responsible for cell cycle progression; and hormones with their effects on energy metabolism (*Michalopoulos GK, et al., 1997; Sakamoto T, et al., 1999; Fausto N, et al., 2006; Malato Y, et al., 2008*) (Figure 1). However, the mechanisms of liver regeneration are still incompletely understood.

1.1.4.1 Cytokine network and the initiation of liver regeneration

The entry of quiescent hepatocytes into the cell cycle, corresponding to the G0/G1 transition, is often defined as “priming” during the first hours after PH. This process is complex and involves the activation of multiple pathways. Immediately after PH, a wide variety of immediate early genes are differentially activated by transcription factors. Cytokines are responsible for activating these genes. Activation of immediate early genes results in a series of events including DNA synthesis, cell replication, and an increase in cell size over several days. These immediate early genes also allow

the liver maintain its essential metabolic functions during the process of liver regeneration. This process occurs in hepatocytes as well as non-parenchymal liver cells, with hepatocyte replication occurring earlier than other cell types (*Michalopoulos GK, 2007*).

Recent studies have pointed out that this initiation of liver regeneration is driven by cytokine release and the innate immune system. An increase in cytokines such as tumor necrosis factor (TNFα), interleukin 6 (IL-6), interleukin 1 (IL-1), and lymphotoxin β (LT-β), which are produced by non-parenchymal liver cells and remnant hepatocytes, is observed. TNFα and IL-6 are considered to be the main participants in the cytokine network which activates liver regeneration.

The cytokine network in the regenerating liver is initiated through the binding of TNFα to the soluble receptor TNFR1 on non-parenchymal cells, primarily Kupffer cells, resulting in multiple events in liver cells. One such event is activation of nuclear factor kappa-B (NF-κB). NF-κB is a ubiquitously expressed transcription factor that controls the expression of cytokine-codifying genes, regulates the cellular cycle and is an antagonist of apoptosis during the liver regenerative process. NF-κB consists of different proteins with distinct biological activities. The active form of NF-κB is retained in the cytoplasm of quiescent cells via binding to inhibitory IκB proteins; activation is induced within 30 min after PH. The activity is transient and disappears after 4-5 hours (*Cressman DE, et al., 1994*).

Another event following binding of TNFα to TNFR is the production of IL-6, which subsequently induces an activation cascade. IL-6 binds to its receptor IL-6R and activates two main pathways through the gp130-IL-6R complex via the signal transducer and activator of transcription 3 (STAT3) and mitogen-activated protein kinase (MAPK) signaling pathways. Binding of IL-6 causes dimerization of the receptor, activation of intracellular tyrosine kinases (JAKs) which phosphorylate gp130 and subsequently dimerize STAT3. Dimerized STAT3 translocates to the nucleus where it regulates the expression of a large number of genes, including those involved in inflammation, the acute phase response, and proliferation (*Dierssen U, et al., 2008*). STAT3 is activated in a slower fashion than NF-κB and is detectable in the liver 1-2 h post-PH; activation lasts for 4-6 h (*Cressman DE, et al., 1995*). Notably, one of the downstream targets of STAT3 is SOCS3, which in turn negatively regulates the expression of IL-6 (*Croker BA, et al., 2003*). IL-6 can also activate MAPK signaling, which is crucial for cellular proliferation, as well as phosphatidylinositol 3-kinase (PI3K)

signaling, which is associated with cell survival (*Yeoh GC, et al., 2007; Levy DE, et al., 2002*).

More evidence that cytokines are important for regeneration has arisen from well-conducted studies on genetically modified animals. These studies found that NF-κB and STAT3 are significantly activated post-PH (*FitzGerald MJ, et al., 1995; Cressman DE, et al., 1995*); administration of anti-TNF antibodies after PH inhibits IL-6 production and DNA replication in a rat model (*Akerman P, et al., 1992*); TNFR-1 and IL-6 deficient mice block liver regeneration induced by PH, accompanied by severely impaired DNA synthesis and failed binding of NF-κB and STAT3 transcription factors shortly after PH (*Cressman DE, et al., 1996; Yamada Y, et al., 1997*). Interestingly, since injection of IL-6 corrects the defect in DNA synthesis in TNFR-1 deficient mice in a PH model, it seems that TNFα mediates liver regeneration depending on its ability to induce the production of IL-6. This has been further demonstrated in studies showing that TNF knockout mice appear to regenerate normally (*Hayashi H, et al., 1995; Fujita J, et al., 2001*). These results can be further explained that TNF itself may not be necessary during liver regeneration, because other ligands can signal through TNFR1, such as lymphotoxin alpha (LTα). Indeed, LTA/TNF double KO mice, which have an LTα and TNFα deficient background, demonstrate inhibited hepatocyte DNA replication (*Knight B, et al., 2005*). Altogether, we it appears that TNFα signaling through TNFR can initiate liver regeneration and acts by activating an IL-6-dependent pathway.

IL-6 accounts for almost 40% of the expression of immediate early genes in the regenerating liver, suggesting that IL-6 has multiple functions, including its role in the acute phase response, hepatoprotection, and mitogenesis (*Taub R, 2004*). A large number of gene-activation pathways are altered in IL-6 deficient mice, which can be employed to explain defective liver regeneration. However, it seems that IL-6 is not the only cytokine involved in the initiation of liver regeneration, because the process is only delayed in the absence of IL-6. In addition, treatment of IL-6 knockout mice with IL-6 induces a much smaller set of genes in the liver which are absent in PH, indicating that IL-6 cooperates with other factors that are induced by PH. Stem cell factor (SCF) and oncostatin M (OSM) have been identified as potential targets or enhancers of IL-6 signaling during liver regeneration. Treatment with SCF can reverse the defective regeneration in IL-6 knockout mice in CCl_4-induced injury model (*Ren X, et al., 2003*). In contrast, IL-6 does not restore the impaired regeneration after CCl_4-induced injury in OSM receptor deficient mice (*Nakamura K, et al., 2004*).

These findings indicate that IL-6, SCF, and OSM may cooperate to activate STAT3 and MAPK signal transduction pathways. Studies in liver-specific STAT3-null mice demonstrate a significant contribution of the IL-6-induced STAT3 pathway to immediate early gene expression (*Li W, et al., 2002*). This observed decline in immediate early gene expression in STAT3-null mice was similar but not identical to the gene expression in IL-6 knockout mice. These results show that STAT3 promotes cell cycle progression and proliferation in vivo, blurring the lines between growth factor and cytokine-regulated pathways. STAT3 is considered as the main IL-6-mediated effector of hepatoprotection; however, the MAPK pathway is normally activated in STAT3-null mice, supporting the theory that not all effects of IL-6 on hepatocyte proliferation are mediated by STAT3.

As a result of the activation of these transcription factors (NF-κB and STAT3), hepatocytes program the response of primary growth (immediate genes, activation of AP-1 and pro-oncogenesis: c-Myc, c-fos, c-Has, c-met, c-Erb). These genes relate directly or indirectly to preparative events for the entry of hepatocytes into the cell cycle. The precise role of these genes expressed early in liver regeneration is not always clear. The early changes in gene expression should be viewed as serving both the entry of hepatocytes into the cell cycle as well the orchestration of specific adjustments that hepatocytes have to make so that they can deliver all essential hepatic functions while going through cell proliferation.

1.1.4.2 Growth factors and cell cycle progression

In addition to cytokine-dependent pathways, several growth factors including the major factors hepatocyte growth factor (HGF), transforming growth factor-alpha (TGFα), and heparin-binding epidermal growth factor like growth factor (HB-EGF) function to promote cellular replication during liver regeneration. This passage involves retinoblastoma (Rb) phosphorylation and increased expression of p107 and cyclins D, E, and A (*Menjo M, et al., 1998; Albrecht JH, et al., 1993*). In addition, cdk4/cyclinD and cdk2/cyclinE complexes are formed.

HGF and its receptor tyrosine kinase Met (c-Met) are considered as the central stimulatory pathway for G1-S progression in remnant hepatocytes. During the initial phase of liver regeneration, both the release of preformed HGF and enhanced gene transcription appear to occur. Inactive, single-chain HGF, which is synthesized mainly by non-parenchymal cells and stored in the extracellular matrix is

rapidly activated by urokinase-type plasminogen activator (uPA), and increased activity of uPA is one of the fast changes induced by PH. This conclusion has been validated by studies showing that blocking uPA delays the appearance of HGF, and followed by delayed liver regeneration, whereas blocking plasminogen-activator inhibitor (PAI) accelerates the release of HGF and thereby accelerates liver regeneration (*Ueki T, et al., 1999; Matsuda Y, et al., 1997*). Activated HGF regulates multiple processes in multiple cell types in the regenerating liver, including motility and tissue development. It acts in a paracrine and endocrine fashion on hepatocytes to promote DNA synthesis and also alters cell morphology and motility. Treatment with HGF antibodies to block its activation results in a blocked regenerative response in CCl_4 injection induced liver injury, and HGF overexpression or exogenous HGF induces hepatocyte proliferation and accelerates the process of liver regeneration after PH in mice (*Bell A, et al., 1999; Sakata H, et al., 1996; Shiota G, et al., 1998; Patijn GA, et al., 1998*). In addition, studies of liver regeneration in c-Met deficiency (the receptor for HGF) were also conducted. Huh et al. concluded that HGF/c-Met signaling is important in hepatoprotection from apoptosis and in facilitating healing after CCl_4 injection (*Huh CG, et al., 2004*). Borowiak et al. reported impaired liver regeneration in mice carrying the Mx-Cre-induced Met mutation, and also demonstrated that activation of Erk1/2 kinase during liver regeneration depends exclusively on Met (*Borowiak M, et al., 2004*). ERK1/2 has been shown to lead to hepatocyte proliferation in vitro and DNA replication in vivo (*Spector MS, et al., 1997*). In summary, these studies have shown that HGF/c-Met signaling pathways are essential for liver regeneration.

The EGF receptor ligand family is another group of mitogens involved in the proliferative phase, which is composed of EGF, TGFα, HB-EGF, and amphiregulin. These various ligands to the EGF receptor are known to activate a phosphorylation cascade that leads to DNA replication and play different and often overlapping functions (*Bor MV, et al., 2006*). Notably, as overlapping functions of different ligands to the EGF receptor exist and it is not currently possible to block all of these ligands simultaneously, it is difficult to demonstrate that a single potent hepatocyte mitogen (TGFα, EGF, or HB-EGF) is crucial for hepatocyte proliferation. For example, TGFα expression increases after PH in wild-type mice, and treatment with TGFα results in constitutive hepatocyte proliferation (*Webber EM, et al., 1994*), although TGFα knockout mice reveal no defects in liver regeneration after PH (*Campbell JS, et al., 2006*). HBEGF is expressed early in the regenerating liver after PH (*Kiso S, et*

al., 2003), preceding the transcriptional increase in HGF and TGFα. Delayed liver regeneration can be observed in HB-EGF knockout mice after PH, although TGFα can act in a compensatory mechanism (*Mitchell C, et al., 2005*).

Auxiliary mitogens include TNF, IL-6, norepinephrine, Notch and Jagged, vascular endothelial growth factor (VEGF), the gene encoding for insulin-like growth factor binding protein (IGFBP), bile acids, serotonin, complement, leptin, estrogens, and fibroblast growth factor (FGF1 and FGF2). A series of knockout models involving these mitogens were demonstrated to delay but not prevent liver regeneration (*Michalopoulos GK, et al., 2010; Lesurte IM, et al., 2006; Desbois-Mouthon C, et al., 2006; Sturm JW, et al., 2004*).

1.1.4.3 Interactions between cytokines and growth factors

Coordinated pattern of gene expression and the existence of overlap suggest an interactive relationship between growth factors and cytokines throughout different phases in the regenerating liver. There are a few signal transduction molecules (for example, ERK and JNK), transcription factors (for example, AP1 and CCAAT-enhancer-binding protein (C/EBPβ)) and other molecules (for example, insulin-like-growth-factor-binding protein (IGFBP-1)), which are involved in the liver regeneration response; these molecules seem to be regulated by both growth factors and cytokines (*Taub R, 2004; Fausto N, et al., 2005*). This combination of cytokines and growth factors might act as a compensatory mechanism for liver regeneration and repair after injury. One important linkage between cytokines and growth factors may be JNK and MAPK-ERK, which can induce cell proliferation and the expression of cyclin D1, and can both be activated by TNFα, HGF, and IL-6 (*Talarmin H, et al., 1999; Coutant A, et al., 2002*). Insulin-like growth factor binding the growth factor and cytokine-mediated pathways protein (IGFBP) might be another linkage, for it may be activated both by IL-6 and HGF. IGFBP modulates cell growth through IGF pathways, or encodes a pro-mitogenic and hepatoprotective protein (*Leu JI, et al., 2001*). Its transcription is partly regulated by IL-6, which accounts for approximate 50% of IGFBP1 gene induction after PH. Although IGFBP1 deficient mice develop normally, liver regeneration after PH is impaired, and is characterized by liver necrosis and reduced and delayed DNA synthesis in hepatocytes (*Weir E, et al., 1994; Leu JI, et al., 2003*). Another possible point of intersection between HGF and IL-6 signals could be the

up-regulation of the various homo- and heterdimeric AP-1 transcription factors, including the Jun-Fos heterodimer. AP-1 activity is required for the activation of a number of proteins that are involved during liver development and regeneration. This conclusion can be validated by the fact that a failure to regenerate is accompanied by increased cell death and lipid accumulation in c-Jun deficient mice after PH (*Behrens A, et al.,* 2002).

1.1.4.4 Termination of liver regeneration

Regenerating liver mass to a particular size is highly regulated and is controlled by the functional needs of the organism after PH. The majority of research surrounding liver regeneration has focused on the initiating phase. Yet, the mechanisms involved in the termination of liver regeneration require critical review because they remain poorly understood.

TGFβ and related TGFβ-family members are identified as the most well-known hepatocyte antiproliferative factors within the liver. TGFβ is produced by stellate cells and is up-regulated in the late phase of liver regeneration in response to signaling from HGF and EGF (*Derynck R, et al., 2003; Michalopoulos GK, et al., 2001*). For now, the specific mechanisms to explain how TGFβ modulates the regenerative process are still not fully established, and the overall data to support TGFβ as the primary stimulus for termination during liver regeneration are still lacking. For example, blockade of the action of either TGFβ or activin leads to the initiation of DNA synthesis in the intact liver, suggesting they may play a critical role in the maintenance of constant liver mass (*Ichikawa T, et al., 2001*). In contrast, a liver-specific TGFβ receptor knockout mice exhibited normal regulation of the termination phase after PH, unless activin A was also eliminated, indicating that activin A may compensate to regulate liver regeneration when signaling through the TGFβ pathway is abolished (*Oe S, et al., 2004*).

During liver regeneration, growth factor and cytokine-regulated pathways are activated, and these pathways have checkpoints that could be feedback-inhibited, thus regulating liver growth and size (*Koniaris LG, et al., 2004*). Suppressors of cytokine signaling (SOCS) are important negative regulators of the cytokine signaling cascade. SOCS3 is rapidly up-regulated by IL-6 signaling, which in turn prevents the phosphorylation of STAT3, thereby terminating the IL-6 signal. This negative feedback loop explains why overexpression of IL-6 can lead to increased liver injury and impaired

cell growth after PH (*Wustefeld T, et al., 2000*). SOCS3 hepatocyte-specific knockout mice demonstrated marked enhancement of DNA replication and liver weight restoration after PH, suggesting an important role in controlling the normal proliferative response in hepatocytes (*Riehle KJ, et al., 2008*). In addition, IL-6 itself could have a role in terminating the HGF signal by inducing plasminogen activating inhibitor (PAI), which blocks the processing of pro-HGF into active HGF (*Shimizu M, et al., 2001*).

In addition, other theories surrounding the termination of liver regeneration have also been established. For example, up-regulation of microRNA (miR) 34a has been reported to contribute to the suppression of hepatocyte proliferation (*Chen H, et al., 2011*); thrombospondin-1 (TSP-1) has been identified as a novel negative regulator of liver regeneration by activating TGFβ (*Hayashi H, et al., 2012*); enhanced liver regeneration in liver-specific ablation of integrin-linked kinase (ILK) mice suggests an essential role in controlling hepatocyte cell cycle in the termination of liver regeneration (*Apte U, et al., 2009*); glypican 3 (GPC3) is reported to play an important role in terminating hepatocyte proliferation and regulating liver size in mice (*Lin CW, et al., 2011*); lipid metabolism and peroxisome proliferator-activated receptor (PPAR) signaling pathways are reported to participate in late-phase liver regeneration (*Yuan X, et al., 2011*); recent work completed by Wuestefeld et al. identified the mitogen-activated protein kinase kinase 4 (MKK4) as a master regulator of liver regeneration, as silencing MKK4 resulted in an increased regenerative capacity of hepatocytes in mouse models of acute and chronic liver disease (*Wuestefeld T, et al., 2013*).

1.1.4.5 Metabolic pathways in liver regeneration

As discussed above, the most proximal events that stimulate liver regeneration and the distal signals that terminate this process remain incompletely understood. Recent data suggest that the metabolic response to hepatic insufficiency might be a proximal signal that initiates regenerative hepatocellular proliferation. Rodents subjected to PH or exposed to hepatotoxic substances develop stereotypical alterations in hepatic and systemic metabolism (*Rudnick DA, et al., 2012*). These changes, which are among the earliest events to occur in response to experimentally induced hepatic insufficiency, begin with marked alterations in hypoglycemia, followed by changes in circulating and hepatic metabolic levels. The functional importance of such changes for liver

regeneration has been implied by several experimental observations. For example, 85% to 90% hepatectomy results in delayed and impaired liver regeneration and increased mortality (*Gaub J, et al., 1984; Lehmann K, et al., 2012*), suggesting that disruption of normal metabolism by resecting a threshold amount of liver mass precedes the onset of regenerative hepatocellular proliferation and resolves with the restoration of normal liver mass. Furthermore, various experimental strategies that suppress specific aspects of these metabolic alterations impair the ensuing hepatic regenerative response; for example, glucose supplementation impairs liver regeneration in liver resection- or hepatotoxin-induced hepatocellular proliferation (*Weymann A, et al., 2009*), and glucose supplementation also suppresses regeneration associated with hepatic fat accumulation (*Holecek M, 1999*). Moreover, suppressing hepatic accumulation pharmacologically (using clofibrate (*Srinivasan SR, et al., 1990*), supraphysiological leptin supplementation (*Shteyer E, et al., 2004*), or propranolol (*Walldorf J, et al., 2010*)) or genetically (as in FLD- or liver-specific glucocorticoid receptor knockout mice) inhibits liver regeneration after PH or other liver injury model (*Gazit V, et al., 2010*). Amino acids also regulate hepatocyte proliferation through modulation of cyclin D1 expression, and administration of amino acids promotes hepatocyte replication, whereas protein restriction impairs regeneration (*Gebhardt R, 1990; Freeman TL, et al., 1999*).

The studies summarized above link alterations in metabolism to the regulation of liver regeneration but, unfortunately, no specific responsible molecular mechanisms have been defined. There are several attractive candidates specifically suggested by experimental analyses of hepatocellular proliferation to connect metabolism and regeneration. For example: (1) PPARα is involved in regulating lipid accumulation. Endogenous lipid metabolites as ligand activators of PPARα raise the possibility that these and other naturally occurring PPARα ligands might link transient hepatic lipid accumulation after PH to subsequent initiation of regenerative hepatocellular proliferation (*Wheeler MD, et al., 2003; Anderson SP, et al., 2002; Chakravarthy MV, et al., 2009*). (2) Farnesoid X receptor (FXR) is a bile acid-activated transcriptional regulator, and global disruption of its expression results in marked impairment of liver regeneration in response to PH (*Zhang L, et al., 2012*). Furthermore, unoperated, bile acid fed mice exhibit increased hepatocellular mitosis and hepatomegaly (*Borude P, et al., 2012*). These observations suggest that the proportionately increased enterohepatic delivery of bile acids to the postresection liver remnant might link altered

metabolism to the initiation of liver regeneration. (3) Mammalian target of rapamycin (mTOR), which is part of a complex that senses nutrient or energy status and also integrates growth factor signals, is considered to be another important complex that may regulate liver regeneration by modulating cell growth and proliferation in response to the energy demands of the remaining liver. Pharmacological inhibition of mTOR suppresses cyclin D1 expression and hepatocellular proliferation in mice subjected to PH (*Huang W, et al., 2006; Goggin MM, et al., 2004*). mTOR is negatively regulated by the ATP/AMP ratio by activating energy sensitive AMP-activated protein kinase (AMPK), and decreased ATP/AMP has been demonstrated as important for the progression of regeneration (*Crumm S, et al., 2008; Hay N, et al., 2004*). (4) EGFR and glycogen synthase kinase 3 (GSK-3) have also been identified to be influenced by metabolism during liver regeneration. Disruption of EGF receptor-dependent signaling might contribute to the inhibitory effect of glucose supplementation on liver regeneration (*Weymann A, et al., 2009*); distinct subcellular pools of GSK-3 exist, with the pool responsive to glycemic alterations and involved in promoting liver regeneration distinct from that which controls β-catenin degradation (*Monga SP, et al., 2001; Jin J, et al., 2009*).

1.2 PPARγ and proliferation

1.2.1 General features of PPARγ

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the nuclear receptor superfamily of ligand -inducible transcription factors (*Dreyer C, et al., 1992*). Similar to typical ligand-activated nuclear receptors, PPARs are comprised of distinct functional domains, including an N-terminal transactivation domain (AF1), a highly conserved DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD) containing a ligand-dependent transactivation function (AF2), which presents potential targets for modulation of the PPARγ signaling cascades (*Poulsen L, et al., 2012*). Three different splice variants of PPARγ have been identified, namely PPARγ1, PPARγ2, and PPARγ3. PPARγ expression is tissue dependent; whereas PPARγ1 is expressed in a broad range of tissues, PPARγ2 is restricted to adipose tissue and PPARγ3 is abundant in macrophages, white adipose tissue and the large intestine (*Fajas L, et al., 1998; He W, et al., 2003;*

Braissant O, et al., 1996). Many studies have revealed that PPARγ controls the expression of networks of genes involved in a large variety of biological processes including metabolism, anti-inflammation, cell cycle and differentiation, as well as immunoregulation by binding to PPAR-responsive regulatory elements as obligate heterodimers with the retinoid X receptor (RXR) (*Lehrke M, et al., 2005; Chang TH, et al., 2000; Kersten S, et al., 2000*). In addition, to date, no functional differences between PPARγ1 and PPARγ2 have been identified.

From the elucidated crystal structure studies of PPARγ, a divergent amino acid sequence in the LBD provides the molecular basis for ligand selectivity. PPARγ is activated by a diverse array of natural and synthetic ligands. However, even though fatty acids and their derivatives (15-deoxy-(12,14)-prostaglandin J2 (15d-PGJ2) (*Forman BM, et al., 1995*) and oxidized metabolites of linoleic acid 9-hydroxy- and 13-hydroxy-octadecadienoic acids (HODE) (*Nagy L, et al., 1998; Tontonoz P, et al., 1998*)) act as natural ligands to activate PPARγ, the identification of specific endogenous ligands is difficult, and thus specific modes of action related to fatty acids and their metabolites have not been clearly defined (*Forman BM, et al., 1996*). For example, it is still not clear if 15d-PGJ activates PPARγ in vivo, and many biological effects of the molecule are clearly independent of PPARγ (*Chawla A, et al., 2001*). Conversely, synthetic ligands such as thiazolidinediones (TZDs, including troglitazone, rosiglitazone, and pioglitazone), have been identified as potent activators of PPARγ with robust insulin-sensitizing activities (*Kung J, et al., 2012*). Evidence that TZDs mediate their insulin sensitizing effects via PPARγ has come from studies that show a strong correlation between the binding affinity of a TZD to PPARγ and its glucose lowering properties in vivo (*Willson TM, et al., 1996*). Additionally, an irreversible synthetic antagonist of PPARγ, GW9662, was also developed, which can inhibit PPARγ activity in cellular transactivation assays and can also inhibit TZD-induced adipocyte differentiation (*Huang JT, et al., 1999*).

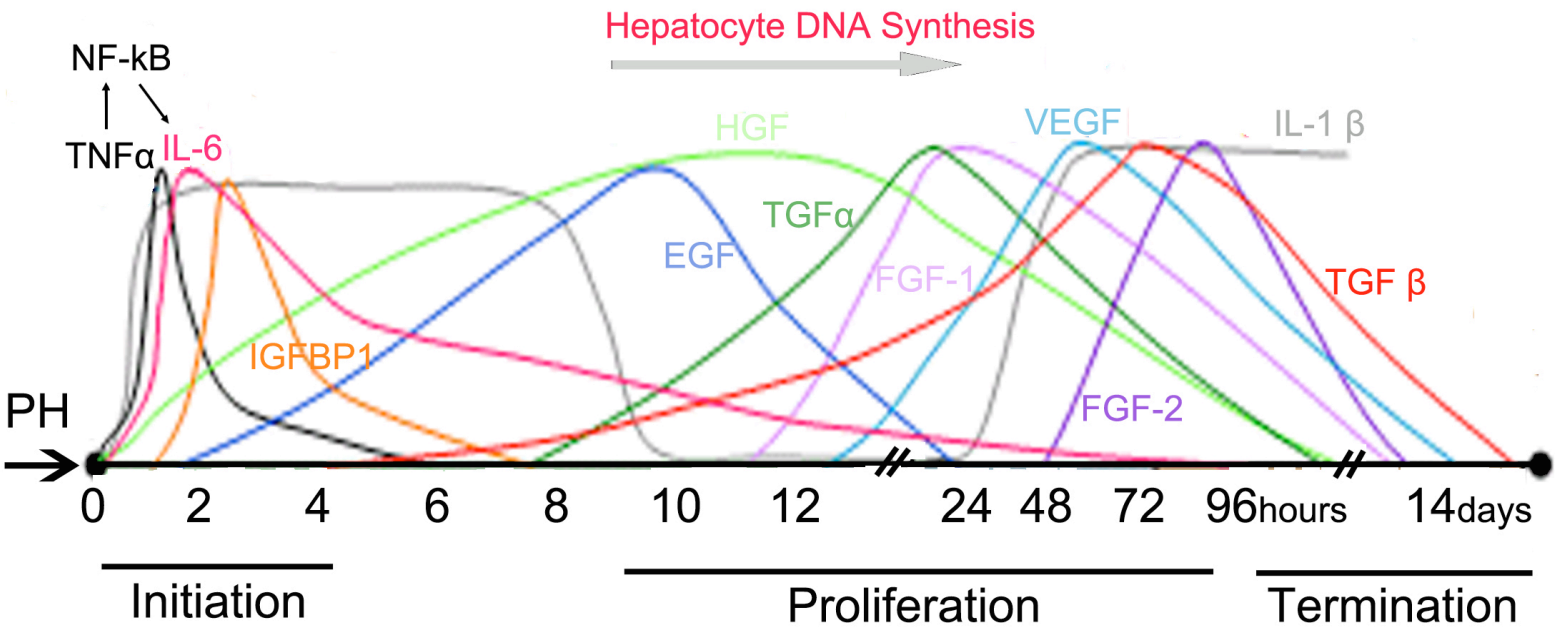


Figure 1. Cytokines, growth factors and co-mitogens involved in different phase of liver regeneration (Modified from Marissa RT et al)

Partial hepatectomy triggers several signals simultaneously in the liver. Gut-derived factors, such as LPS, activate hepatic non-parenchymal cells (including Kupffer cells and stellate cells) and increase the production of TNFα and IL-6. TNFα and IL-6 activate neighboring hepatocytes, which causes STAT3 activation and the expression of stem-cell factor (SCF) and several proteins; subsequently, this results in activated growth factor-mediated pathways, including vascular endothelial growth factor (VEGF), which binds to endothelial cells, and triggers the release of pro-HGF from stellate cells, which can be cleaved to release HGF. HGF binds to the Met receptor on hepatocytes to activate the PI3K, AKT and S6 kinase signal transduction pathways. HGF signaling releases TGFα and other downstream signals that are also shared with the cytokine-mediated pathway, such as AP1, JNK, pERKs, C/EBPβ and IGFBP1. These factors are proposed to activate target of rapamycin (mTOR) and lead to cell-cycle transition by increasing the expression of cyclins D and E and reducing p27 levels. Cooperative signals from these factors promote DNA synthesis and hepatocyte proliferation. Various inhibitory proteins, including TGFβ, PAI, SOCS3 and p27 and other cyclin-dependent kinase inhibitors are blocked during the proliferative phase but restored at the end of the process of regeneration by helping to return hepatocytes to the quiescent state.

A number of coregulator molecules have been identified, including steroid receptor coactivator1 (SRC-1), CREB-binding protein (CBP/p300), the tuberous sclerosis gene-2 product, the PPAR binding protein, PPAR-gamma coactivator 1 and 2(PGC-1 and PGC-2), and Ara70 to act for multiple regulated functions in some settings. The functions of coactivators for PPARγ include enhancing the transcriptional activation of PPARγ-regulated genes and participating in the formation of a well-known multisubunit protein complex (the TRAP/DRIP/ARC/mediator complex) to play an important role in connecting CBP/p300 bound coactivators (*Viswakarma N, et al., 2010*).

Activation of PPARγ plays an inhibitory role in cell proliferation and growth by virtue of its differentiation inducing ability. The precise mechanisms linking the modulation of PPARγ with growth inhibition has not been elucidated. PPARγ ligands exert their effects through both PPARγ dependent and independent pathways, often triggering crosstalk with other signaling pathways. A better understanding of the biological role of PPARγ and its ability to trigger crosstalk with other cell signaling pathways would allow for rational development of selective PPARγ modulators and for targeting aspects of PPARγ biology that are implicated in tumor progression (Figure 2).

1.2.2 PPARγ crosstalk with cytokines

PPARγ is expressed in different kinds of cells, including monocyte/macrophages, endothelial cells, vascular smooth muscle cells, T-cells, and B-cells, and mediate effects on cell survival, surface-protein expression, and cytokine and chemokine expression depending on the cell type (*Bishop-Bailey D, et al., 1999*). Especially, it has been extensively reviewed elsewhere that PPARγ can inhibit the ability of certain cytokines to induce inflammatory responses. PPARγ ligands inhibit the induction of inducible nitric oxide sythase (iNOS), matrix metallopeptidase (MMP-9), and scavenger receptor A gene transcription, and the production of TNFα, IL-1β, and IL-6. Furthermore, activated PPARγ will directly bind corepressors of cytokine-induced pro-inflammatory transcription factors AP-1, making them unfit for nuclear translocation. This process shuts down the transcriptional activity of the aforementioned transcription factors and may nullify the symptoms of inflammation (*Ricote M, et al., 1999; Szanto A, et al., 2008*). Notably, antagonism between PPARγ and the metabolic/pro-inflammatory cytokine TNFα is the most well-established evidence to suggest the role for PPARγ in regulating the expression of cytokines (*Jiang C, et al., 1998; Ruan H, et al.,*

2008). In addition, it is important to note that many studies also report that PPARγ has no anti-inflammatory activity or might indeed exert a pro-inflammatory response. Furthermore, the anti-inflammatory effects of TZDs still remain in PPARγ knockout mice, suggesting a PPARγ-independent mechanism of agonist activation (*Moore KJ, et al., 2001*). This independent mechanism was further investigated; it was found that the PPARγ ligand 15d-PGJ2 inhibits the secretion of TNFα and IL-6 in macrophages stimulated by bacterial lipopolysaccharide, and mediates anti-inflammatory effects by directly binding and inactivating I-κB kinase (*Castrillo A, et al., 2000*). Furthermore, 15d-PGJ2 and rosiglitazone are potent inducers of SOCS proteins and the overexpression of either SOCS1 or SOCS3 prevents both JAK-2 as well as STAT3 phosphorylation in primary astrocytes (*Park EJ, et al., 2003*). The mechanism of PPARγ ligands in regulating cytokines still needs to be further clarified.

Recent observations also suggest that PPARγ function also can be suppressed by cytokines. Treatment with TNFα or IL-1 inhibits the ligand-induced transcriptional activity of PPARγ, and this suppression is mediated through NF-κB activated by the TAK1/TAB1/NF-κB-inducing kinase (NIK) cascade, a downstream cascade associated with IL-1 and TNFα signaling. NF-κB blocks PPAR-gamma binding to DNA by forming a complex with PPARγ and its AF-1-specific co-activator PGC-2 (*Suzawa M, et al., 2003*).

1.2.3 PPARγ crosstalk with growth factors

Growth factor binding to their specific receptors and inducing the initial events of mitogen-activated protein kinase (MAPK) cascade activation are essential in cell cycle regulation. It seems that MAPK acts as an intermediary to link PPARγ and growth factors. Next, we will focus on reviewing the interaction between MAPK signaling and PPARγ, so that we can get some hints of the crosstalk between PPARγ and growth factors.

Given the role of PPARγ as an inducer of adipocyte differentiation, it is likely that this receptor could be involved in the regulation of signaling pathways that lead to the cessation of cell growth. PPARγ can inhibit MAPK, preventing MAPK from phosphorylating and thereby activating downstream transcription factors, which are necessary for MAPK-dependent pro-inflammatory gene expression and cell progression (*Park JY, et al., 2009; Ji H, et al., 2010*). On the other hand, several studies

have implicated a negative role for growth factor induced MAPK activation in regulating PPARγ function. It was demonstrated that ERK, JNK, and p38 MAPKs significantly inhibit ligand-dependent independent and transcriptional activation of PPARγ through phosphorylating a consensus-MAPK motif (PXSPP) located in the N-terminal AF-1 domain (*Adams M, et al., 1997; Hu E, et al.,1996*). Consistently, mutating the growth factor activated MAPKs phosphorylation motif in PPARγ prevents its phosphorylation as well as growth factor-mediated transcriptional repression (*Camp HS, et al., 1997*). Apart from this, upon mitogenic stimulation, phospho-MEK can directly interact with PPARγ without significantly phosphorylating PPARγ. This leads to rapid export of the PPARγ-MEK complex from the nucleus to the cytoplasm through the nuclear export signal of MEK, thus reducing the transcriptional activity of PPARγ (*Burgermeister E, et al., 2007; Burgermeister E, et al., 2007*). Various in vivo studies have also established that regulation of PPARγ by growth factor activated MAPK cascades is involved in controlling the balance between proliferation and differentiation in certain cell types. For example, cooperation between PPARγ and MEK1 facilitates the adipogenic program by MEK1-dependent induction of the C/EBP gene during adipogenic differentiation of mesenchymal stem cells (*Prusty D, et al., 2002; Chuang CC, et al., 2007*). This was further validated in vivo as mice with mutant negative regulation of the transactivation activity of PPARγ exhibit resistance to diet-induced obesity. Interestingly, MAPK signaling can also modulate PPARγ functions by regulating the expression of coactivator 1 (PGC1) needed for PPARγ transcriptional activation (*Coll T, et al., 2006*).

1.2.4 Activation of PPARγ antagonizes TGFβ signaling

Published studies have shown that TGFβ binding to its receptor induces phosphorylation of R-Smads (Smad2 and Smad3) that subsequently translocate into the nucleus to regulate the transcription of target genes, particularly regulators of cell proliferation and extracellular matrix production (*Attisano L, et al., 2002*). Although the exact mechanism remains to be defined, the mutual interference between PPARγ and TGFβ signaling pathways has been reported at multiple levels, including phosphorylation of PPARγ, repression of PPARγ gene expression, and the interaction between PPARγ and Smad3 (*Reka AK, et al., 2010*). In hepatobiliary cells, overexpression of PPARγ blocks TGFβ-induced Smad transcriptional activity and mitoinhibition. In these cells, TGFβ treatment simultaneously activates Smad-mediated gene transcription and

phosphorylation of cPLA2α, whereas phosphorylation of cPLA2 initiates two signaling pathways that counteract Smad-mediated growth inhibition, including activation of its G-protein-coupled receptor EP1 through PGE2 and activation of PPARγ. Depletion of cPLA2 or PPARγ enhances TGFβ-mediated Smad activation and partially restores the growth inhibition by TGFβ (*Han C, et al., 2004*). On the other hand, during adipocyte differentiation, PPARγ activity is inhibited by TGFβ by repressing the transcriptional activity of C/EBPs, which are important co-regulators of PPARγ, but not repressing the transcriptional activity of PPARγ (*Choy L, et al., 2003*). Such a mutually antagonistic affect between PPARγ and TGFβ needs to be further investigated. Inconsistent with the above findings, in vascular smooth muscle cells (VSMCs), PPARγ activation by pioglitazone increased TGFβ levels and translocation of phospho-Smad2 into the nucleus and thus induced apoptosis to exert direct anti-atherosclerotic and anti-restenotic effects. These effects were eliminated either by using the PPARγ antagonist GW9662 or by silencing, demonstrating that TGFβ is a downstream effector of PPARγ (*Redondo S, et al., 2005*). In addition, accumulating evidence suggests that the activation of PPARγ can also interfere with TGFβ signaling in the tumor microenvironment. Both natural and synthetic PPARγ agonists are reported to suppress the activation of fibroblasts into myofibroblasts, which usually occurs via TGFβ signaling in advanced stages of cancer. The PPARγ ligands 15d-PGJ2, ciglitazone, and rosiglitazone inhibited TGFβ-driven myofibroblast differentiation as well as type I collagen production in human lung fibroblasts without affecting their viability (*Burgess HA, et al., 2005*). Pioglitazone attenuates the induction of fibronectin and its spliced variant EDA+FN by TGFβ in human mesangial cells (*Maeda A, et al., 2005*). Similarly, pioglitazone counteracts fibronectin activated invasion of breast carcinoma through the suppression of TGFβ signaling (*Moustakas A, et al., 2007*).

1.2.5 PPARγ in metabolism

The number of genes that are directly and indirectly regulated by PPARγ contribute to its abundant functions. These targets contribute to the important role of PPARγ in regulating lipid and glucose metabolism. Consistent with its central role in lipid metabolism, PPARγ is mainly expressed in adipose tissue and is associated with adipocyte differentiation, self-renewal and maintaining the normal function of mature adipocytes (*Barak Y, et al., 1999; Tang W, et al., 2011; Imai T, et al., 2004*). This has been demonstrated by studies on the PPARγ-null mouse that was completely

devoid of adipose tissue. PPARγ is expressed in a newly identified adipocyte progenitor within the white adipose tissue perivascular niche, suggesting that it may contribute to adipocyte self-renewal; ablation of PPARγ in the mature adipocytes of mice induced adipocyte death, suggesting PPARγ is required for mature adipocyte function. In addition to its role in lipid metabolism, PPARγ is also crucial for maintaining glucose homeostasis. Ligand activation of PPARγ by rosiglitazones was found to increase the expression and translocation of the glucose transporters GLUT1 and GLUT4 to the cell surface, thus increasing glucose uptake in adipocytes and muscle cells and reducing glucose plasma levels (*Standaert ML, et al., 2002; Kramer D, et al., 2001*).

As is well-known, insulin is the most potent physiological anabolic agent, promoting the storage and synthesis of lipids, proteins, and carbohydrates and inhibiting their breakdown and release into the circulation (*Saltiel AR, et al., 2001*). Synthetic agonists of PPARγ (TZDs) have shown a promoting effect on lipid uptake and storage in adipose tissue, thereby lowering systemic free fatty acid (FFA) levels and reducing FFA delivery to other tissues, where they have been implicated in inducing insulin resistance (*Shulman GI, 2000*). This finding suggests that activation of PPARγ can increase adipocyte insulin sensitivity, which may be mediated in part by direct activation of genes encoding factors of the insulin signaling pathway (*Ribon V, et al., 1998; Smith U, et al., 2001*). Consistently, in the patients with Type 2 diabetes, that activation of PPARγ by TZDs decreases glycated hemoglobin (HbA1c) and fasting and postprandial glucose and lowers circulating insulin levels; this was also considered largely as a consequence of an improvement in insulin sensitivity. Some studies have been conducted to investigate the underlying mechanism linking PPARγ with insulin sensitivity. PPARγ activation can increase the expression of intracellular proteins such as c-Cbl-associated protein (CAP), which is predominant in insulin-sensitive tissues and correlates well with insulin sensitivity, to modulate the insulin signal transduction pathway (*Ribon V, et al., 1998*). Moreover, PPARγ activation regulates the production of adiponectin, resistin, and TNFα, and these cytokines can impact insulin sensitivity (*Rangwala SM, et al., 2003*). Significantly, considering that these factors probably act through distinct signaling pathways and different, although overlapping, tissue targets, several different mechanisms may be involved to explain the effect of PPARγ on insulin sensitivity.

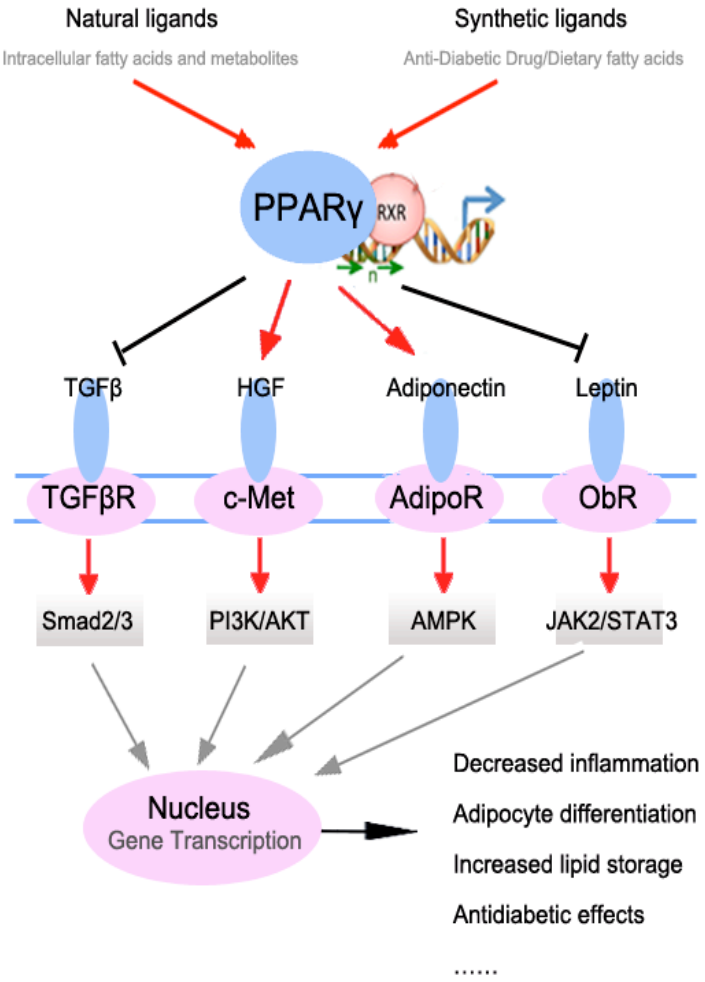


Figure 2. Schematic diagram of PPARγ signaling pathways.

PPARγ is activated by synthetic or endogenous ligands and subsequently binds RXR to affect the transcription of target genes which are involved in regulating insulin sensitivity, adipogenesis and placental function. PPARγ activates transcription in concert with coactivators including SRC1, CBP/p300, the tuberous sclerosis gene-2 product, the PPAR binding protein, PGC-1, PGC-2, and Ara70. The activity of PPARγ is also regulated by the growth factor-induced MAPK cascade.

1.3 PPARγ in modulating liver regeneration

In recent years, several studies have determined that PPARγ is involved in tumorigenesis and development, as it leads to cell cycle arrest, promotes cell differentiation, inhibits angiogenesis, and induces apoptosis (*Michalik L, et al., 2006; Heaney AP, et al., 2003; Koga H, et al., 2001; Schaefer KL, et al., 2005*). The cell cycle regulation properties of PPARγ, along with its role in cell apoptosis, have encouraged pursuing new functions in liver regeneration. Some studies have shown that activation of PPARγ by thiazolidinediones (TZDs) can inhibit liver regeneration (*Turmelle YP, et al., 2006; Yamamoto Y, et al., 2008*). In addition, a more recent study revealed that the metabolic and

hepatocellular proliferative response to PH are modestly augmented in liver-specific PPARγ null mice (*Gazit V, et al., 2012*). These observations indicate that PPARγ does play an important role during liver regeneration, although the underlying molecular mechanisms remain unclear. These findings fascinated us and encouraged the evaluation of the underlying mechanism of PPARγ on liver regeneration after surgical resection.

2 AIMS

Liver regeneration is triggered and regulated by a variety of different factors; among them, PPARγ seems to play a major role due to its control over the expression of networks of genes involved in a large variety of biological processes. Organ-directed support/mediation of the regenerative process is of major clinical interest and might be a potential therapeutic application in the future. The goal of this thesis was to increase our understanding of how PPARγ expression is regulated and what impact altered expression of PPARγ might have on the liver.

In particular, we aimed to investigate the expression profile of PPARγ during liver regeneration, the potential regulatory effect of regeneration-induced factors on the expression of PPARγ, and finally to explore the underlying mechanism. The goals of this study are summarized as follows:

• Determine the role of PPARγ in liver regeneration induced by partial hepatectomy in mice.

• Analyze the impact of liver injury associated factors, known to play a central role in liver regeneration, on the expression of PPARγ.

• Investigate the role of PPARγ in knockout mice and the impact on cellular growth and transformation.

• Explore the potential molecular mechanism of PPARγ involved in liver regeneration.

3 MATERIALS & METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

Table 1. Chemicals and reagents

Chemicals and reagents	Supplier
2-Mercaptoethanol	Sigma-Aldrich
6×DNA Loading Dye	Thermo Scientific
Acrylamide Solution	ROTH
Agarose	ROTH
Agarose	ROTH
Ammonium Persulfate (APS)	Sigma-Aldrich
Bovine serum Albumin (BSA)	ROTH
Citric Acid	ROTH
DAB+Chromogen (50×)	DAKO
DreamTaq™ Green PCR Master Mix	Fermentas
ECL Detection Reagent	Amersham
EDTA	ROTH
Eosin	Sigma-Aldrich
Ethanol	Hauseigene Apotheke
Fenofibrate	Sigma-Aldrich
Formamide	Merck Biosciences
GeneRuler™ DNA Ladder	Thermo Scientific
Glycine	ROTH
GW9662	Sigma-Aldrich
Hematoxylin	Merck Biosciences
Hydrochloric Acid	Hauseigene Apotheke
Hydrogen Peroxide (30%)	ROTH
Isoflurane	CP-Pharma, Burgdof
Isopropanol	ROTH
Liquid Nitrogen	Tec-Lab
Methanol	Merck Biosciences
Milk Powder Blotting Grade	ROTH
MOPS	ROTH
Mounting Medium	DAKO
Nitrocellulose Membranes	Bio-Rad
Normal Goat Serum	DAKO
NuPAGE LDS Sample Buffer (4×)	Invitrogen
NuPAGE Sample Reducing Agent (10×)	Invitrogen
PageRuler TM PlusPrestained Protein Ladder	Thermo

Paraformaldehyde (PFA)	Apotheke TU München
Phosphate Buffered Saline (PBS) pH7.4	PAA
Potassium Chloride (KCL)	Merck Biosciences
Protease Inhibitor Cocktail Tablet	ROTH
RIPA Buffer	Cell Signaling Technology
RNAse DNAse-free Water	Invitrogen
Rosiglitazone	Glaxo SmithKline
Roticlear	ROTH
SDS Ultra Pure	ROTH
SGX523	Selleck
Sodium Chloride	Merck Biosciences
Sodium Citrate	Merck Biosciences
Sodium Hydroxide	ROTH
Sodium Phosphate	Merck Biosciences
TEMED (Tetraethylmethylenediamine)	Serva
Tris Base	Merck Biosciences
Triton 100×	ROTH
Tween 20	Merck Biosciences

3.1.2 Kit systems

Table 2. Kits

Kit	Supplier
BCA Protein Assay Kit	Thermo Scientific
QIAGEN DNA Mini Kit	Qiagen
QIAquick Purification Kit	Qiagen
QuantiTect Reverse Transcription Kit	Qiagen
RNeasy Plus Mini Kit	Qiagen
SYBR Green Master Kit	Roche Diagnostics
BCA Protein Assay Kit	Thermo Scientific
QIAGEN DNA Mini Kit	Qiagen

3.1.3 Laboratory equipment

Table 3. Laboratory equipment

Laboratory equipment	Supplier
Centrifuge	Eppendorf
Electrophoresis power supply ST606	Gibco
High-quality Precision Balance	KERN
IKA Shakers Vortex Mixer	NeoLab
KL 200 Microscope Standard Illumination System	Zeiss
Leica RM2255 Rotary Microtome	Leica

Light Cycler480	ROCH
Microscope Axiovert 40CFL	Zeiss
Milli-QR Advantage	Millipore
Mini Rocker MR-1	Peqlab
Mini-PROTEANR Electrophoresis System	BioRad
Multipipette Plus	Eppendorf
Multiskan EX	Thermo Scientific
NanoDrop 2000 Spectrophotometer	Thermo Scientific
PCR Machine	VWR
pH-Meter WTW Series	Inolab
Pipetboy	IBS Integra Bioscience
Scanner CanonScan 4400F	Canon
Standard Power Pack P25	Biometra
Thermomixer Comfort	Eppendorf
Titramax 100	Heidolph
Trans-Blot SD Wet Transfer Cell	BioRad
UniProtect Air Flow Cabinet	BioScap
Centrifuge	Eppendorf
Electrophoresis power supply ST606	Gibco
High-quality Precision Balance	KERN
IKA Shakers Vortex Mixer	NeoLab
KL 200 Microscope Standard Illumination System	Zeiss
Leica RM2255 Rotary Microtome	Leica

3.1.4 List of antibodies

Table 4. Antibody list for IHC

Antibody	Catalog No	Dilution	Supplier
Primary antibody			
Rabbit Anti-Ki67 pAb	ab15580	1:400	Abcam
Rabbit Anti-Phospho-Histone H3 (Ser10) pAb	9701	1:500	Cell Signaling Technology
Secondary antibody			
Goat HRP-labelled Anti-Mouse pAb	K4001		Dako Deutschland GmbH
Goat HRP-labelled Anti-Rabbit pAb	K4003		Dako Deutschland GmbH

Table 5. Antibody list for Western blots

Antibody	Catalog No	Dilution	Supplier
Primary antibody			
Mouse Anti-β-actin mAb	Sc-69879	1:5000	Santa Cruz
Mouse Anti-Cyclin D1 mAb	2926	1:1000	Cell Signaling Technology

Rabbit Anti-Cyclin B1 pAb	4138	1:1000	Cell Signaling Technology
Rabbit Anti-44/42 MAPK mAb	9102	1:1000	Cell Signaling Technology
Rabbit Anti-Phospho-Specific-p44/42 MAPK	9101	1:1000	Cell Signaling Technology
Mouse Anti-HGF(7-2) mAb	NBP1-19182	1:200	Novus Biologicals
Mouse Anti-Met(L41G3) mAb	3148	1:1000	Cell Signaling Technology
Rabbit Anti-Phospho-Met (Tyr1234/1235) (3D7)	3129	1:1000	Cell Signaling Technology
Rabbit Anti-GAPDH (14C10) mAb	2118	1:2000	Cell signaling
Mouse Anti-PPARγ (E-8) mAb	sc-7273	1:400	Santa Cruz
Rabbit Anti-PPARα pAb	Ab8934	1:500	Abcam
Rabbit Anti-STAT3 pAb	9132	1:1000	Cell Signaling Technology
Rabbit Anti-Phospho-STAT3(Tyr705) pAb	9131	1:1000	Cell Signaling Technology
Rabbit Anti-p38 MAPK pAb	9212	1:1000	Cell Signaling Technology
Rabbit Anti-Phospho-p38 MAPK	9211	1:1000	Cell Signaling Technology
Rabbit Anti-NF-κB p65 (C22B4) mAb	4764	1:1000	Cell Signaling Technology
Rabbit Anti-Phospho-NF-κB p65 (Ser536) pAb	3031	1:1000	Cell Signaling Technology
Secondary antibody			
Sheep HRP-labelled Anti-Mouse IgG Ab	NA931	1:5000	GE Healthcare (Little Chalfont,
Donkey HRP-labelled Anti-Rabbit IgG Ab	NA934	1:5000	GE Healthcare (Little Chalfont,

3.1.5 Primers

Table 6. Oligonucleotide primers for qRT-PCR

	Sense	Anti-sense
IL6	5'-TCCTCTCTGCAAGAGACTTCCATCC-3'	5'-CCTCTGTGAAGTCTCCTCTCCGG-3'
TNFα	5'-TCGGGGTGATCGGTCCCCAA-3'	5'-TGGTTTGCTACGACGTGGGCT-3'
Cyclin D1	5'-GCTGTCTTGCACTCTGGTGT-3'	5'-CTGCGCTTGGAGTGATAGAA-3'
Cyclin B1	5'-AGGCTGCTTCAGGAGACCATGT-3'	5'-TGGCCGTTACACCGACCAGC-3'
β2M	5'-CCAGAAAACCCCTCAAATTCA AG-3'	5'-AGTTCAGTATGTTCGGCTTCCC-3'

3.2 Methods

3.2.1 Animal protocols

3.2.1.1 Treatment of wild-type mice

Eight to ten week-old female C57BL/6J mice (Charles River laboratory) weighing around 20 g were maintained under a standard 12-hour-light/dark cycle with free access to standard mouse chow and tap water before and after surgery. In order to evaluate the effect of PPARγ on liver regeneration in the PH mouse model, we treated mice with 20 mg/kg body weight rosiglitazone (Glaxo SmithKline) by oral gavage or 10 mg/kg body weight GW9662 (M6191, Sigma Aldrich) by intraperitoneal injection 2 days before surgery. To further investigate the underlying mechanism of PPARγ on liver regeneration, two other groups of mice were treated with 25 mg/kg body weight SGX523 (Selleck) by oral gavage beginning on the operative day or a concentration of 0.2% fenofibrate mixed with chow food beginning 5 days before PH. All treatments continued through to the time of animal sacrifice and tissue harvest.

3.2.1.2 Generation of PPARγ $^{fl/fl}$ Albcre^{+} mice

Conditional hepatocyte-specific PPARγ knockout mice were generated by mating Albcre transgenic mice. PPARγ LoxP/LoxP mice lacking the Cre transgene were used as control animals.

3.2.1.3 Partial hepatectomy model

70% PH was performed as described previously (*Mitchell C, et al., 2008*), under general anesthesia with inhaled isoflurane (n=4~6 for each time point in each treatment group). Briefly, 70% PH was achieved by ligation and extirpation of the left and median lobes after mid-ventral laparotomy. The mortality rate was less than 5%. For sample collection, necropsy was carried out immediately after anesthesia. Removed liver lobes were immediately weighed, flash-frozen in liquid nitrogen and stored at -80°C for subsequent genomic and proteomic analysis.

All animal experimental procedures were carried out under a protocol approved by the animal studies committee and were in accordance with institutional guidelines.

3.2.2 Biochemical analysis

Transaminases like alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) activity, glucose, triglyceride and cholesterol were determined by the Rechts der Isar hospital clinical laboratory.

3.2.3 Histology and immunohistochemistry

3.2.3.1 Preparation, fixation and permeabilization of slides

Liver tissue was fixed overnight in 4% PFA, embedded in paraffin, and sectioned at 3 µm. The slides were deparaffinized in Roticlear I, II, III one after another for 10 min respectively at RT and rehydrated in graded concentrations of ethyl alcohol (EtOH) (100%, 96%, 90%, 80%, 70%) for 2 min every step, then rinsed in distilled water for 5 min.

3.2.3.2 Hematoxylin and eosin staining (HE staining)

After rehydration, the prepared slides were stained in hematoxylin for 30 seconds and then rinsed in running tap water for 15 min. Afterwards, slides were counterstained in eosin for 5 seconds. The slides were washed in tap water and dehydrated by in ascending alcohols with a final immersion in Roticlear three times. Finally, the slides were mounted with Vectashield mounting medium.

3.2.3.3 Immunohistochemistry (IHC)

Immunohistochemistry was performed using the Dako Envision System following the listed steps.

· To enhance the antigen retrieval, sections were treated with citrate buffer in the microwave for 10 min, cooled down and washed in TBS three times for 5 min.

· Blocked with 3% peroxidase which was diluted with fresh methanol for 10 min and washed in in TBS three times for 5 min.

· Blocked with TBS/3% BSA for 1 h at room temperature.

· Diluted primary antibody to different concentration in TBS/3% BSA and pipetted it onto the slides and incubated overnight at 4°C.

· Rinsed the slides three times with TBS/0.1% BSA and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 45 min, then washed for three times with TBS/0.1% BSA.

· Performed the enzymatic reaction with substrate solution (0.5 mg DAB/phosphate buffer) and stopped in ddH_2O.

· Rinsed in water and counterstained in Mayer's hematoxylin solution and subsequently rinsed for 10 minutes in running tap water for bluing.

· Dehydrated in graded concentrations of EtOH (95%, 95%, 100%, 100%, 100%) for 2 min respectively and cleared in Roticlear I, II, III (10 minutes).

· Mounted with Cytoseal in fume hood.

Hepatocyte proliferation was determined by Ki67 and PH3-positive cells; the percentage of proliferative hepatocytes was determined by examination of at least four random 200× fields in five different sections.

3.2.4 Protein biochemical methods

3.2.4.1 Protein extraction from liver tissue

All liver tissue lysates were made from snap frozen liver using RIPA buffer (Cell Signaling Technology). A piece of frozen liver tissue (about 100 mg) was put into a sterile tube containing a steel ball, then 300-500 µl of RIPA lysis buffer containing protease inhibitors was added into the tube. The tissue was disrupted using a homogenizer for 3 min, then the samples were placed on ice for 45 min. Samples were centrifuged at 13,000 rpm for 20min at $4^{O}C$, then the upper solution was transferred to a new sterile 1.5 ml Eppendorf tube. Extracted proteins were used immediately for protein determination or stored at $-20^{O}C$.

3.2.4.2 Determination of protein concentration

For protein quantification, the BCA Protein Quantification Kit was used. BCA reagent was freshly prepared by adding 4% $CuSO_4$ to the protein solution at a ratio of 1:50. 10 µl of the probe or the standard were added to a microtiter 96-well plate and mixed with 200 µl of the prepared BCA solution. After incubation at $37^{O}C$ for 25 min, the extinction was measured and the protein

concentration was calculated.

3.2.4.3 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

For SDS-PAGE, the protein samples were mixed with loading buffer and denatured at 95^{O}C for 3 min.

Preparation of protein samples:

Protein	Variable (up to 20 µg)
Lysis Buffer	16.25 µl-volume of protein
Nu page LDS Sample Buffer 4x	6.25 µl
Nu page Reducing Agent 10x	2.5 µl
Total volume	25 µl

Protein samples were separated according to their size by using a discontinuous gel system, which was composed of stacking (5%) and separating gel (7.5-10%) layers which differed in their salt and acrylamide concentrations. Stacking and separating gels were prepared one day prior to use and stored at 4^{O}C. The next day, 20 µg protein were loaded onto a polyacrylamide gel and separated by gel electrophoresis (BioRad, Germany) in running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3) at 80 V until the sample focused in the stacking gel and then at 100 V until the dye ran off the gel.

3.2.4.4 Immunoblotting

After separation by SDS-PAGE, proteins were transferred electrophoretically onto a polyvinylidene fluoride (PVDF) microporous membrane using a Trans-Blot SD Wet Transfer Cell (Bio-Rad). The transfer conditions were 200-300 mA for 1-2 h at RT depending on the molecular weight of the target protein. Steps were then performed as follows:

	Dilution/Solution	**Duration**
Blocking	5% non-fat dry milk or BSA/TBS (1x)	1 hour
Application of primary antibody	Variable/5% non-fat dry milk or BSA	Overnight
Washing	0.05% TBST	3 x 10 min

Application of secondary antibody	1:5000/5% non-fat dry milk	Shaking for 1 hour
Washing	0.05% TBST	3 x 10 min

Signal detection was performed using SuperSignal West Pico Chemiluminescent Substrate according to Thermo-Scientific's instructions. The blots were exposed to an autoradiography film (Hyperfilm ECLTM, Amersham) for 5 sec to 30 min depending on the signal intensity.

3.2.5 Nucleic acid methods

3.2.5.1 RNA-related methods

3.2.5.1.1 RNA isolation

Total RNA was extracted from collected mouse liver tissue using an RNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. Briefly, less than 30 mg of liver tissue was disrupted and homogenized in the appropriate volume of Buffer RLT Plus. After homogenization, the lysate was centrifuged for 3 min at maximum speed at a temperature higher than 20°C, then the supernatant was carefully transferred and centrifuged to remove the genomic DNA. The flow-through was mixed with 1 volume of 50% ethanol, applied onto RNeasy Mini Spin Columns and centrifuged at 8,000 g for 15 sec, then washed by adding RW1 buffer and centrifuged at 8,000 g for 15 sec, then re-washed twice with RPE buffer. Afterwards, the silica-gel membranes were dried by centrifugation of the columns at maximum speed for 1 min followed by elution of total RNA with 20-30 µl RNase-free water and centrifuging for 1 min at maximum speed. The purity and quantity of RNA was determined by a spectrophotometer. Purified total RNA was either stored at -80°C or was used immediately to prepare cDNA.

3.2.5.1.2 Reverse transcription and cDNA synthesis

QuantiTect Reverse Transcription Kit from Qiagen was used to synthesize single-stranded cDNA from total RNA.

· 1 µg of total RNA was added in to 14 µl genomic DNA elimination reaction on ice.

Genomic DNA elimination reaction

Component	volume	Final concentration
gDNA without buffer 7x	2 µl	1x
Template RNA	Variable (up to 1µg)	
RNase-free water	Variable	
Total volume	14µl	

· Reaction system was incubated for 2 min at 42°C, then placed immediately on ice.

· Template RNA was added to each tube containing the reverse transcription master mix.

Reverse-transcription master mix

Component	volume	Final concentration
Quantiscript reverse transcriptase	1 µl	
Quantiscript RT buffer	4 µl	1x
RT primer mix	1 µl	
Entire genomic DNA elimination reaction	14 µl	
Total volume	20 µl	

· Incubated for 15 min at 42°C

· Incubated for 3 min at 95°C to inactivate Quantiscript reverse transcriptase.

· To prevent it from binding to the cDNA, the reaction was immediately cooled on ice for at least 5 min. The synthesized cDNA was immediately used for PCR or stored at -20°C until use.

3.2.5.1.3 qRT-PCR

The LightCycler experiments were performed with the hot-start DNA Master SYBRGreen I kit and using the LightCycler® apparatus (Roche, Germany) following the manufacturer's instructions. The primers used in the PCR reaction were generated by Metabion. For each gene analyzed, a 5 µl aliquot of cDNA was added to a reaction mixture containing gene-specific primers (Table 6), and SYBR Green I Master Mix (Roche). The real-time quantitative PCR was performed using a LightCycler® 480 real-time PCR machine (Roche). The relative amounts of mRNA were determined

by means of the $2^{\Delta\Delta CT}$ method. Amplification was followed by melting curve analysis to verify the correctness of the amplicon. A negative control without cDNA was run with every PCR to assess the specificity of the reaction. Analysis of data was performed using LightCycler software version 3.5. The slope of the standard curve was an indicator of the amplification efficiency. Standard curves were subsequently used to calculate the relative abundance of each transcript in each sample. The measurements were performed in triplicate. All results are represented as mean ± SD. The specificity of PCR products was confirmed by melting curves and electrophoresis.

Reaction Mix

SYBR® GreenER qPCR SuperMix	12.5 µl
Primers(sense primer + antisense primer)	2 µl
cDNA template	5 µl
ddH_2O	3 µl
Total	25 µl

PCR program for all real-time primer pairs

Procedure	Temperature	Duration	
1. Activation	55 °C	2 min	
2. Denaturation	94 °C	10 min	
3. Denaturation	94 °C	15 sec	40 cycles
4. Annealing Tm	55 °C	30 sec	
5. Elongation	72 °C	30 sec	
6. Elongation	72 °C	10min	
6. Conservation	4 °C		

3.2.5.2 DNA-related methods

3.2.5.2.1 DNA isolation

DNA was extracted from collected mice tails using a DNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. A suitable piece of tissue was placed in a 1.5 ml tube, then digested in 500 µl STE buffer and 15 µl proteinase K in a 55°C incubator overnight. After incubation, the samples were centrifuged at maximum speed for 10 min, and the supernatant was transfered into a

new tube and 400 µl of isopropanol was added to each probe to precipitate the DNA. After 10 min of incubation at room temperature, the supernatant was centrifuged for another 10 min at maximum speed. The supernatant was discarded and the remaining DNA pellet was washed with 70% ethanol. The tube was dried before resuspension of the DNA. 50 µl of DNase-free water was then added. The purity and quantity of DNA was determined by a spectrophotometer. The DNA samples were stored at -20°C.

STE Buffer	
NaCl	0.1 M
Tris-HCl	10 mM
EDTA	1 mM
SDS	1%
pH	8
Aqua dest	Various

3.2.5.2.2 Genotyping of PPARγ-transgenic mice

Individual mice genetically modified at the PPARγ locus or transgenic for Cre recombinase were genotyped using a PCR approach according to the following protocols.

Reaction Mix

PCR Master Mix, 2x (Invitrogen)	12.5 µl
Sense primer (100 µM)	0.5 µl
Antisense primer (100 µM)	0.5 µl
DNA template	1 µl
RNase-free water	10.5 µl
Total volume	25 µl

PCR program for genotyping

Procedure	Temperature	Duration	
1. Activation	55 °C	2 min	
2. Denaturation	95 °C	3 min	
3. Denaturation	95 °C	15 sec	35 cycles
4. Annealing Tm	54 °C	30 sec	
5. Elongation	72 °C	30 sec	

6. Elongation	72 °C	2 min
6. Conservation	4 °C	

The oligonucleotides used in the PCR analysis of the transgenic mouse strains were:

PPARγ^{tm2Rev}

oIMR 1934	5'-TGT AAT GGA AGG GCA AAA GG-3'
oIMR 1935	5'-TGG CTT CCA GTG CAT AAG TT-3'

Tg (Alb-cre) 21 Mgn-Alternate 1

20240	5'-TTG GCC CCT TAC CAT AAC TG-3'
oIMR 5374	5'-GAA GCA GAA GCT TAG GAA GAT GG-3'

3.2.6 Statistics

All experiments were performed in triplicate, and the data shown are representative of consistently observed results. Quantitative data are presented as mean±SD. Multiple comparisons were performed by one-way analysis of variance (ANOVA) with repeated measures, followed by the least significant difference Fisher's post hoc test. Statistical significance was set at $P<0.05$. All statistics were performed using SPSS 13.0 (SPSS Inc., Chicago, IL).

4 RESULTS

4.1 Ligand activation of PPARγ inhibits hepatocellular proliferation during mouse liver regeneration

4.1.1 Expression pattern of PPARγ in response to partial hepatectomy

To investigate the role of PPARγ in regulating hepatocyte proliferation, we treated the mice with a PPARγ agonist (rosiglitazone) or antagonist (GW9662) to influence activation of PPARγ activity before PH. The expression pattern of PPARγ in response to PH was first examined. In the liver of control mice, immunohistochemistry (IHC) revealed strong staining in hepatocytes, especially in the nuclei, and occasionally in non-parenchymal cells at the 0 h time point. The distribution of PPARγ in the liver has specific characteristics in that PPARγ is predominantly expressed in the centrilobular zone but weakly in the periportal zone. Following PH, the expression of PPARγ was significantly reduced during the early phase of liver regeneration (1/2-2 days following PH) and gradually recovered during the late phase of regeneration (3-7 days following PH) (Figure 3A). Protein expression of PPARγ by western blot showed a consistent tendency with the IHC results (Figure 3D, E, left panel). Compared to the control group, the expression of PPARγ was maintained during the entire process of regeneration in the rosiglitazone-treated mice (Figure 3B, D) and decreased gradually in the GW9662-treated mice (Figure 3C, E).

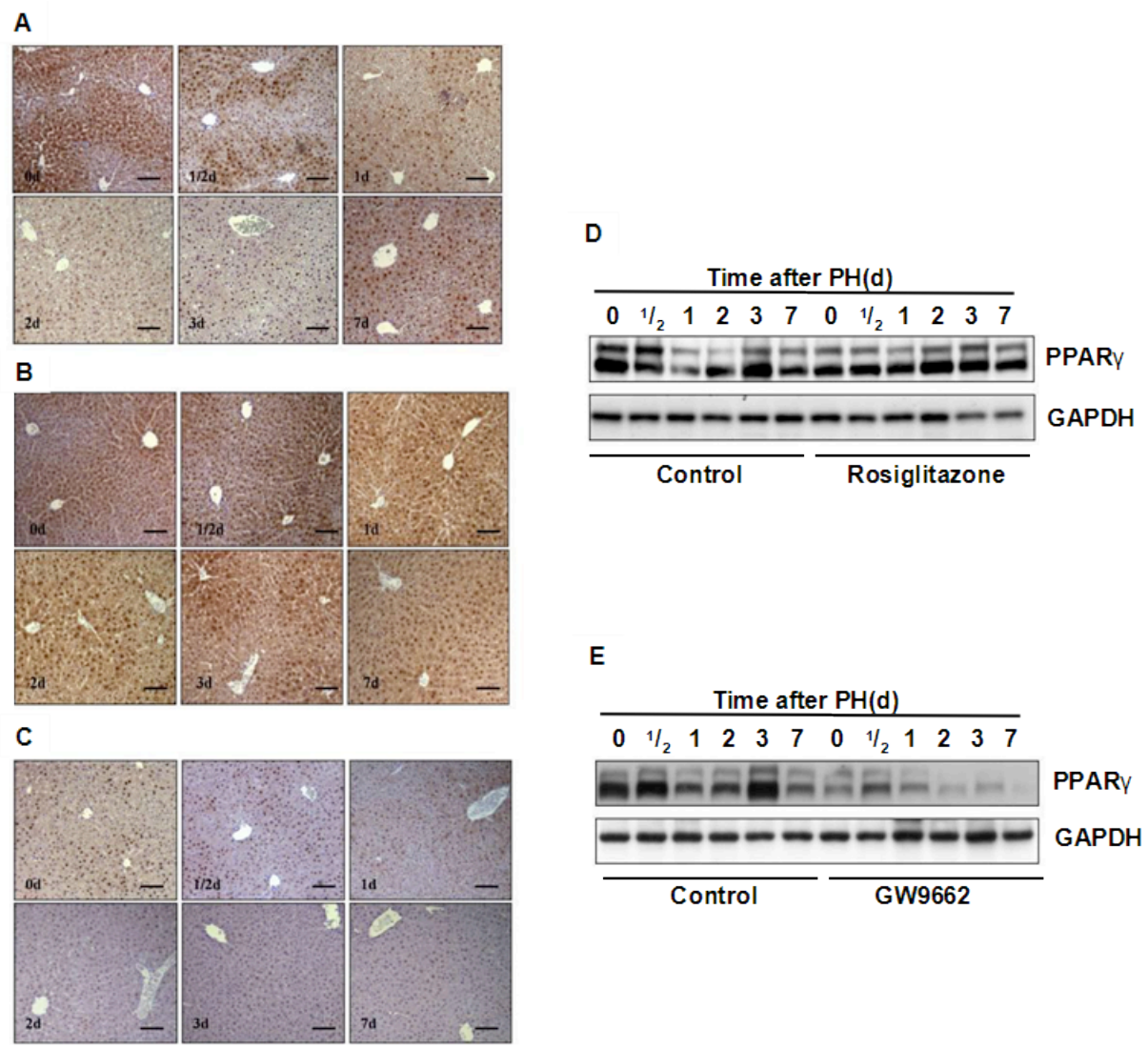


Figure 3. Hepatic PPARγ expression during mouse liver regeneration.

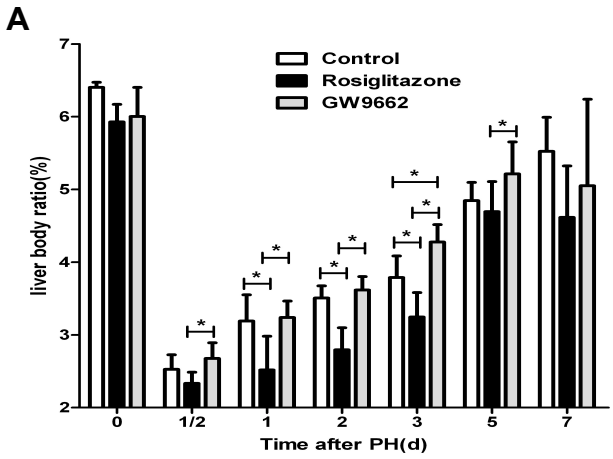
Representative immunohistochemistry (IHC) for PPARγ in control mice **(A)**, rosiglitazone-treated mice **(B)** and GW9662-treated mice **(C)** at different time points after PH. Scale bar: 200 μm. (**D, E)** Hepatic expression of PPARγ protein in control mice, rosiglitazone-treated mice and GW9662-treated mice at different time points after partial hepatectomy.

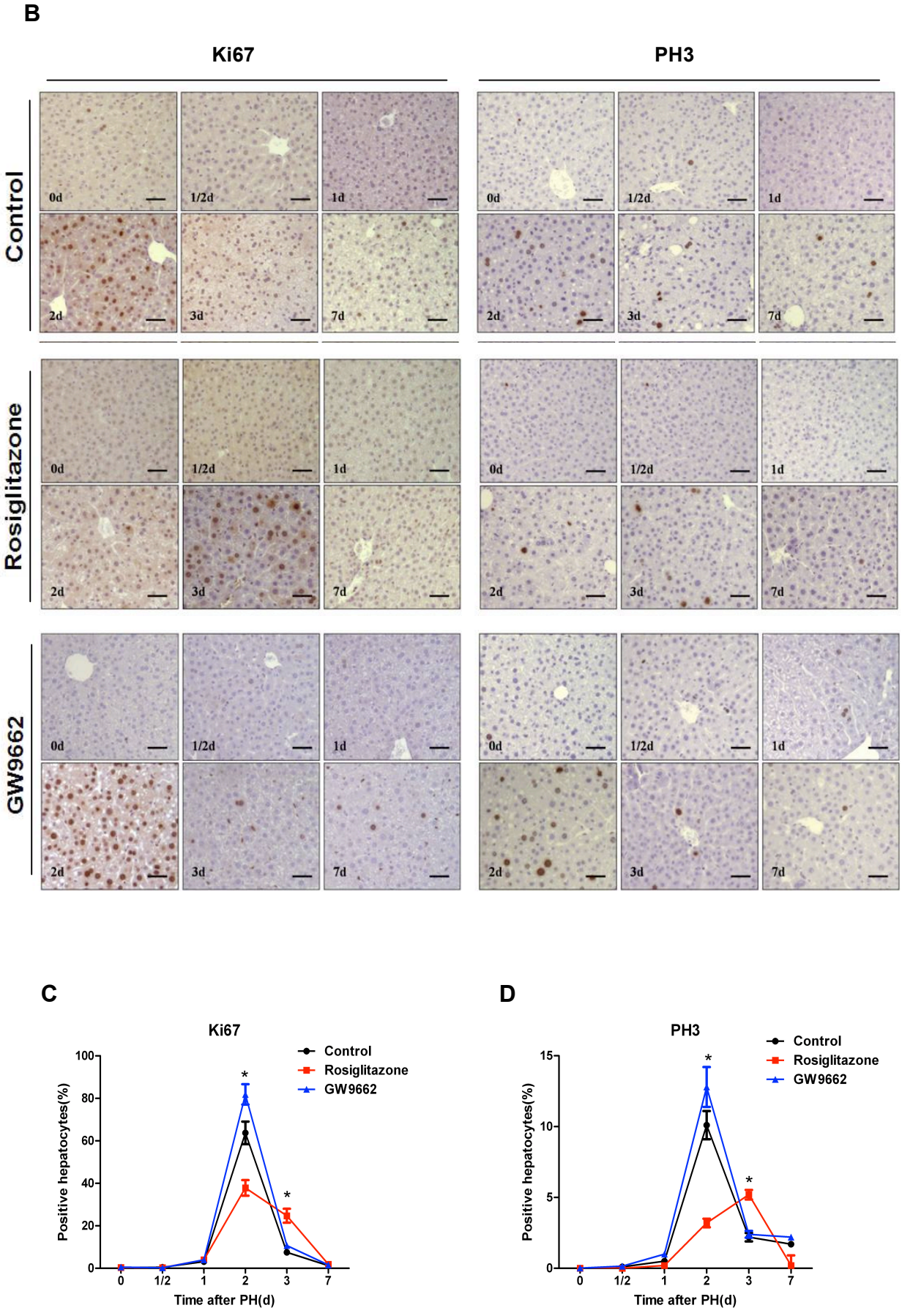
4.1.2 Effect of altered activation of PPARγ on liver regeneration

We next evaluated PH-induced liver regeneration in control, rosiglitazone-treated, and GW9662-treated mice. The liver regeneration ratio (the weight of remnant liver divided by the initial body weight) rose sharply between 1 to 3 days (from 3.19% to 3.79%), and nearly regained the preoperative value at day 7 after PH in the control group. In contrast, rosiglitazone-treated mice displayed significantly delayed regain in liver mass compared to control mice on day 1, 2, 3 post-PH,

whereas the increase was slightly accelerated in GW9662-treated mice on day 3 post-PH (Figure 4A). Despite showing a delayed/accelerated rate of liver regeneration, the final average mass of the remnant livers in each group was not significantly different 14 days after surgery.

To further investigate the effects of PPARγ on hepatocyte proliferation and cell cycle entry in response to PH, we performed IHC for Ki67 and PH3 as well as western blot/qRT-PCR for cyclins in liver tissue. Consistent with previous studies (*Espeillac C, et al., 2011*), the results show that Ki67-positive and PH3-positive hepatocytes peaked at day 2 post-PH in control mice. Interestingly, number of Ki67 and PH3 positive hepatocytes was decreased and delayed in rosiglitazone-treated mice but significantly increased in GW9662-treated mice at day 2 post-PH (Figure 4B, C, D). Correspondingly, the induction of cyclin D1, a key mediator of cell cycle progression at the G1 and G1/S phases of the cell cycle, was attenuated at 12 h post PH in the rosiglitazone-treated mice, as compared to control and GW9662-treated mice (Figure 4E, F). Consistently, the expression of cyclin B1, which was induced by 2 days after PH to regulate G2/M phase cell cycle progression, was less activated at the mRNA level in rosiglitazone-treated mice at the 2 day time point, although no statistical significance was found between GW9662-treated mice and control (Figure 4E, G). Taken together, these data demonstrate that PPARγ activation inhibits liver regeneration at least partly by controlling hepatocyte proliferation.




B
Ki67
PH3
Control
Rosiglitazone
GW9662
0d
1/2d
1d
2d
3d
7d
C
Ki67
Control
Rosiglitazone
GW9662
Positive hepatocytes(%)
Time after PH(d)
D
PH3
Control
Rosiglitazone
GW9662
Positive hepatocytes(%)
Time after PH(d)

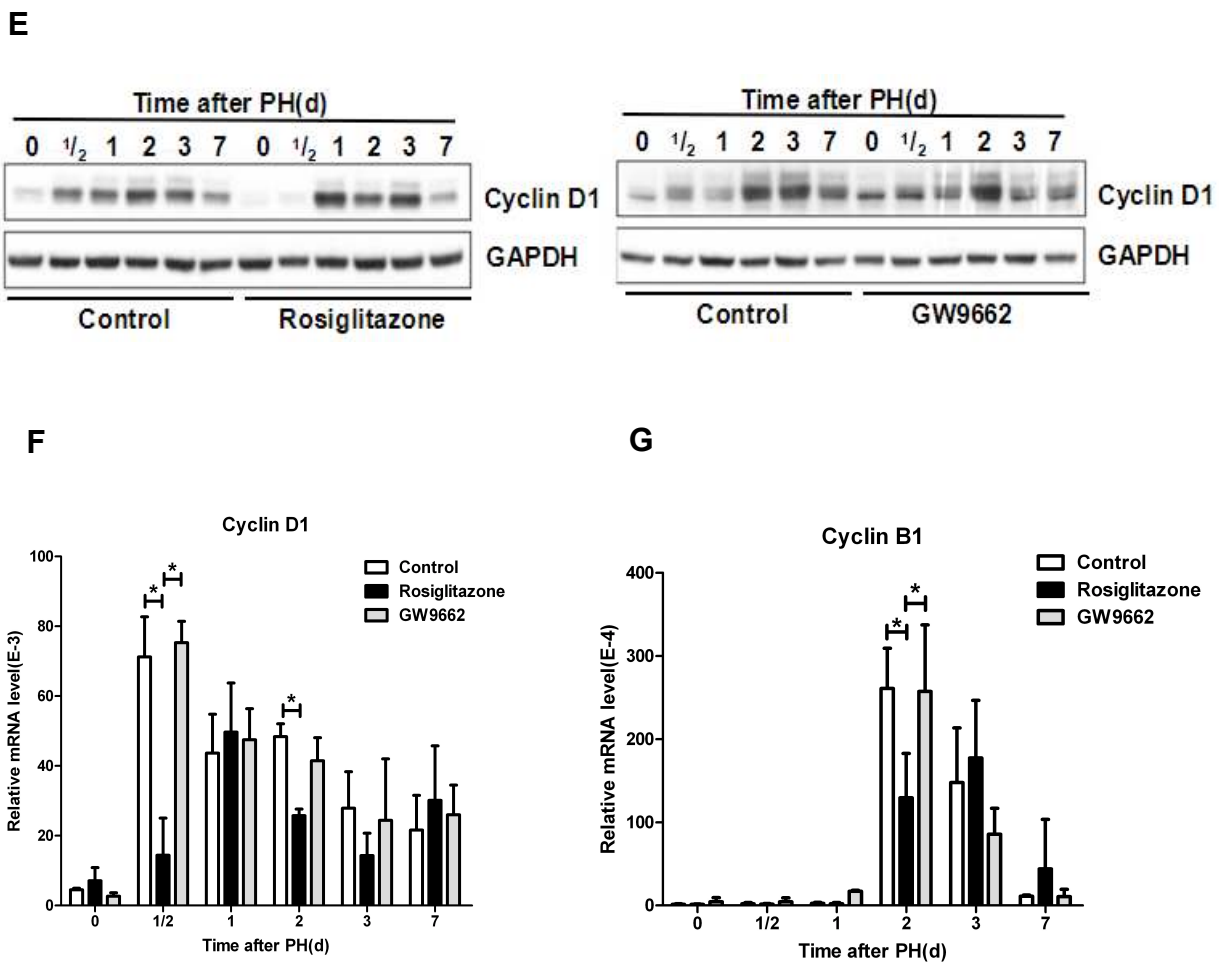


Figure 4. Ligand activation of PPARγ inhibits mouse liver regeneration after PH.

A) Liver to body weight ratio in control mice, rosiglitazone-treated mice and GW9662-treated mice at different time points after PH. **B)** Micrographs of liver sections immunostained with Ki67 and PH3 antibodies from three different groups after partial hepatectomy. Scale bar: 200 μm. **C,D)** Quantified number of Ki67-positive hepatocytes and PH3-positive hepatocytes. **E)** Assessment of cyclin D1 and cyclin B1 protein expression in the regenerating liver. Left panel: Western blot analysis of cyclin D1 and cyclinB1 in control mice vs. rosiglitazone-treated mice. Right panel: control mice vs. GW9662-treated mice. **F,G)** qRT-PCR analysis of cyclin D1 and cyclin B1 mRNA expression after PH in control, rosiglitazone-treated, GW9662-treated groups. * $p<0.05$.

4.1.3 Hepatotoxicity of drug administration

The effects of rosiglitazone and GW9662 on liver histology and plasma aminotransferase levels were also examined to establish the specificity of the activity of PPARγ on hepatic regeneration. HE staining and serum biochemistry analysis revealed that following 9 days (corresponding to the 7 d

post-hepatectomy time point of drug administration), no mouse exhibited histological alterations or abnormal metabolic parameters suggesting drug-induced hepatic injury (Figure 5A, B). These data indicate that the suppressive effects of rosiglitazone on liver regeneration are unlikely to be secondary to drug-induced hepatotoxicity.

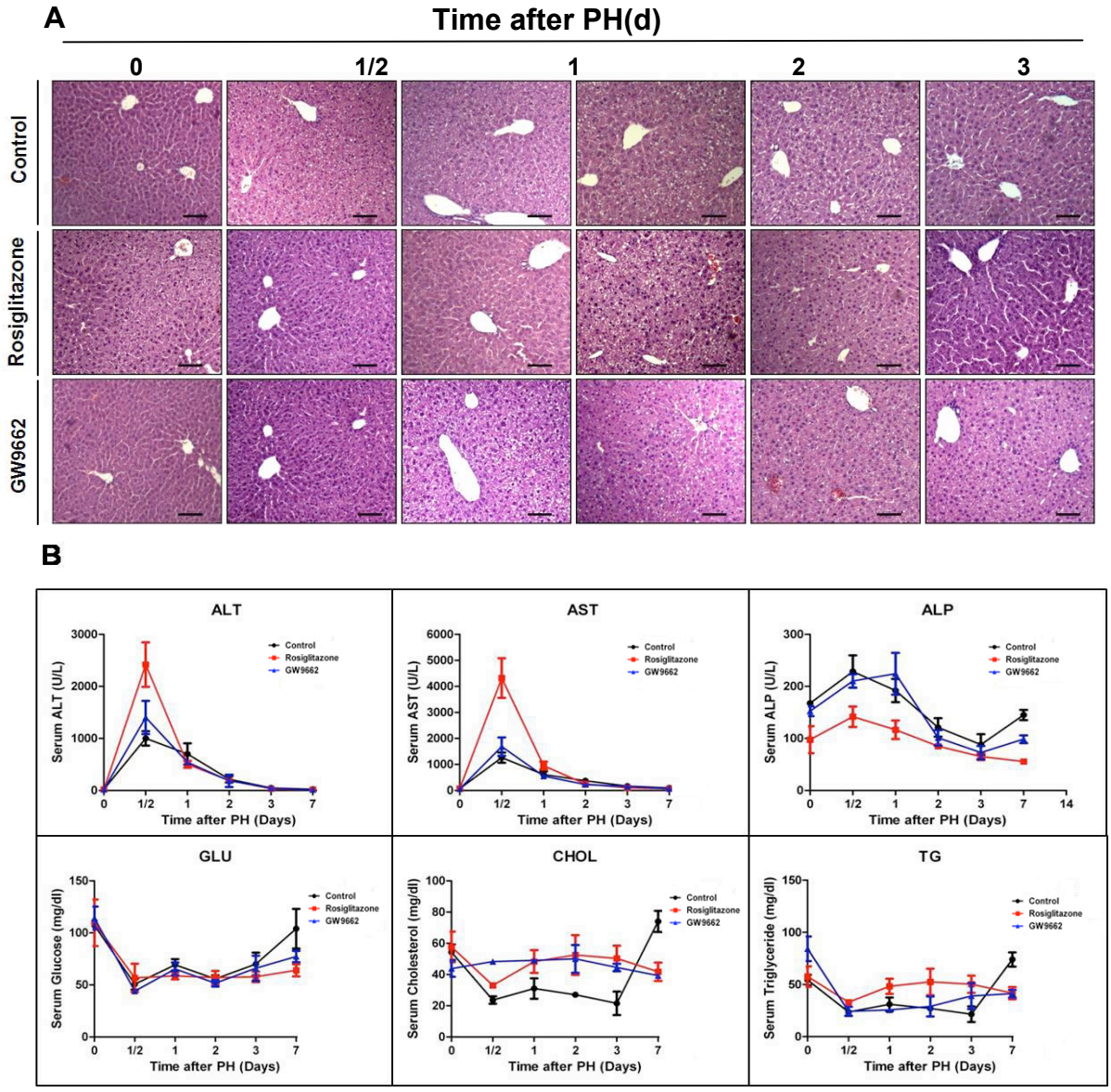
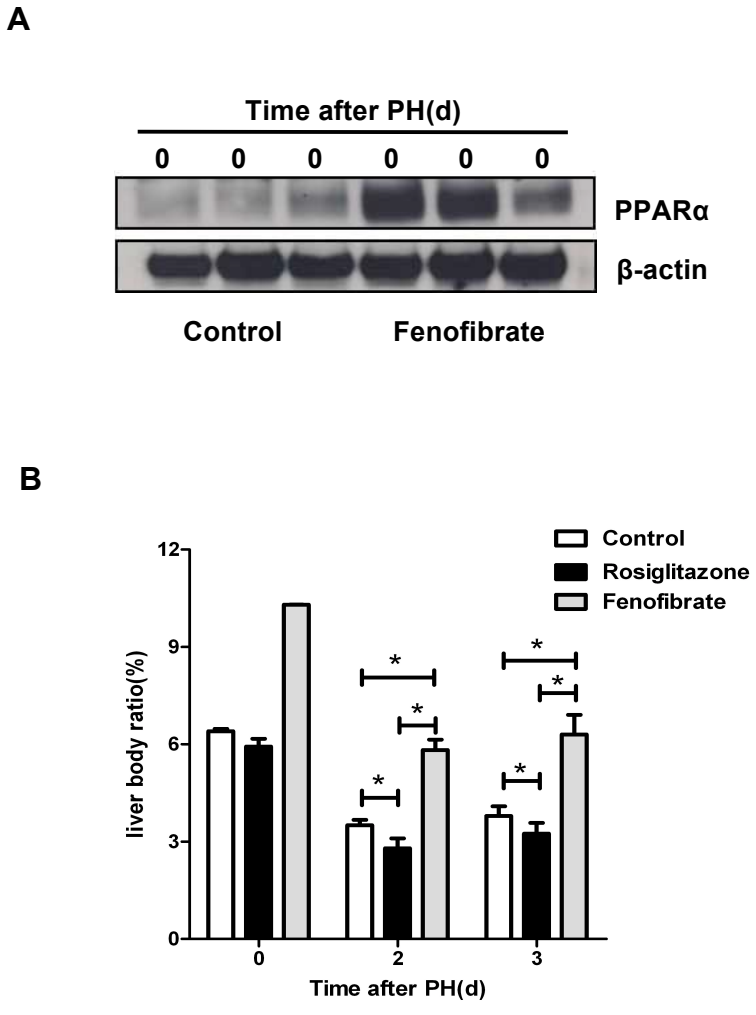


Figure 5. Morphology and systemic metabolic changes during liver regeneration in mice.

A) H&E staining for regenerated liver. Scale bar: 200 μm. **B)** Serum biochemistry analysis to check function of regenerated liver.

4.2 PPARα activation does not influence hepatic regeneration following partial hepatectomy

Peroxisome proliferator-activated receptors (PPARs) are composed of three isoforms (α, β, γ). To address whether rosiglitazone-activated PPARγ was specific in influencing hepatocyte proliferation after PH, fenofibrate treatment was performed to activate another PPAR isoform (PPARα). As shown in Figure 6A, expression of PPARα was robustly up-regulated in wild-type mice 5 days after fenofibrate treatment. However, compared to control mice, up-regulation of PPARα had no influence on the number of Ki67 and PH3-positive hepatocytes (Figure 6C, D, E, F) at the 0, 2, and 3 day time point after PH in fenofibrate-treated mice. These results indicate that, unlike rosiglitazone, fenofibrate-activated PPARα in the liver is not sufficient to regulate liver regeneration in the PH model.



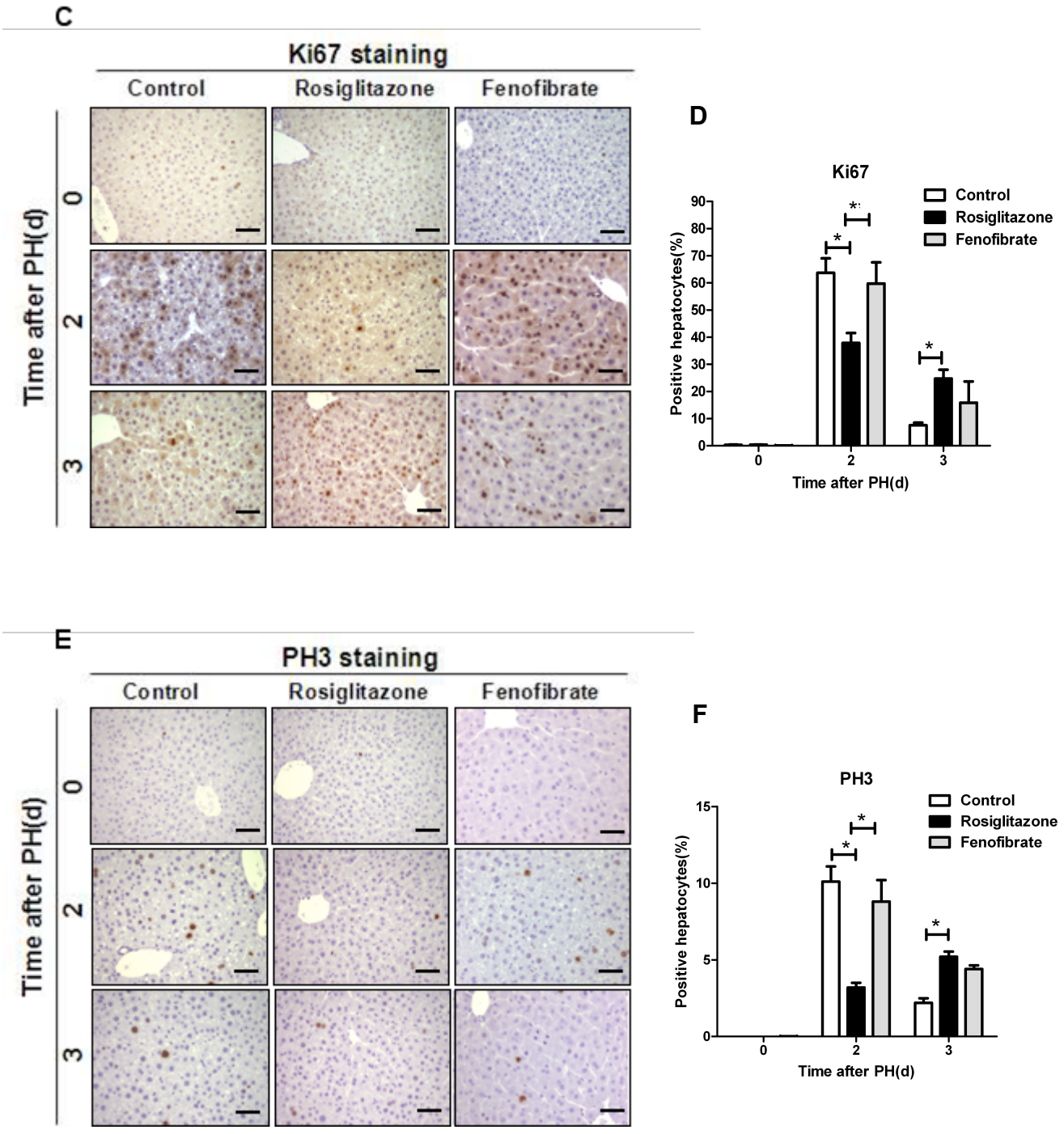


Figure 6. Effect of PPARα agonist Fenofibrate on liver regeneration.

A) Western blot to demonstrate the efficiency of the fenofibrate method. **B)** Liver to body weight ratio in control mice, rosiglitazone-treated mice and fenofibrate-treated mice at different time points after PH. **(C, E)** IHC for hepatic Ki67 and PH3 after hepatectomy. **(D, F)** Number of Ki67-positive hepatocytes and PH3-positive hepatocytes. * $p<0.05$.

4.3 PPARγ inhibits liver regeneration by regulating cytokines and growth factors

4.3.1 PPARγ does not impair the initiation of mouse liver regeneration

To identify the mechanistic role of PPARγ in liver regeneration, the effect of rosiglitazone on signaling events associated with liver regeneration were evaluated. During liver regeneration, the TNFα-IL-6 signaling pathway plays a pivotal role in maintaining the viability of hepatocytes and enhancing their responsiveness to growth factors. Since PPARγ activation inhibits TNFα production in macrophages, we first examined whether PPARγ activation inhibits liver regeneration via decreased TNFα/IL-6 production. The hepatic mRNA levels of TNFα and IL-6 were increased 12 h after PH in all control, rosiglitazone-treated and GW9662-treated mice; however, at both time points (0 and 12 h), no significant difference was observed among these different groups (Figure 7A, B). Similarly, obvious induction of phosphorylated STAT3 was observed 4 h after surgery in all groups, but the magnitude and kinetics of IL-6-induced STAT3 phosphorylation were comparable among control, rosiglitazone-treated and GW9662-treated mice at the early stage of liver regeneration (0-12 h) (Figure 7C). Collectively, these results indicate that altered expression of PPARγ is dispensable for the control of TNFα-IL-6-STAT3 signaling pathway during initial liver regeneration.

Next, we determined the effects of rosiglitazone on p38 MAP kinase activation, which is considered to be essential for normal regeneration. Our results show that a reduction in the relative phosphorylation p38 MAP kinases at 2-4 h after PH in both rosiglitazone-treated mice and control mice. However, compared to the control group, neither accelerated nor delayed activation of p38 MAP kinases was observed in rosiglitazone-treated mice.

The transcription factor NF-κB, consisting of the subunits RelA/p65 and p50, is known to be quickly activated after PH. Current concepts suggest that activation of NF-κB is especially critical in non-parenchymal cells to produce cytokines (TNF, IL-6) in order to adequately prime hepatocytes to proliferate. We also evaluated the effect of rosiglitazone on the activation of p65. The results show that changes in p65 phosphorylation depended on the time point after PH in rosiglitazone-treated group, similar to what was observed in the control group. Taken together, ligand activation of PPARγ does not influence the initial activation of cytokines or subsequent cytokine-induced activation of STAT3, NF-κB, and p38 MAP kinase.

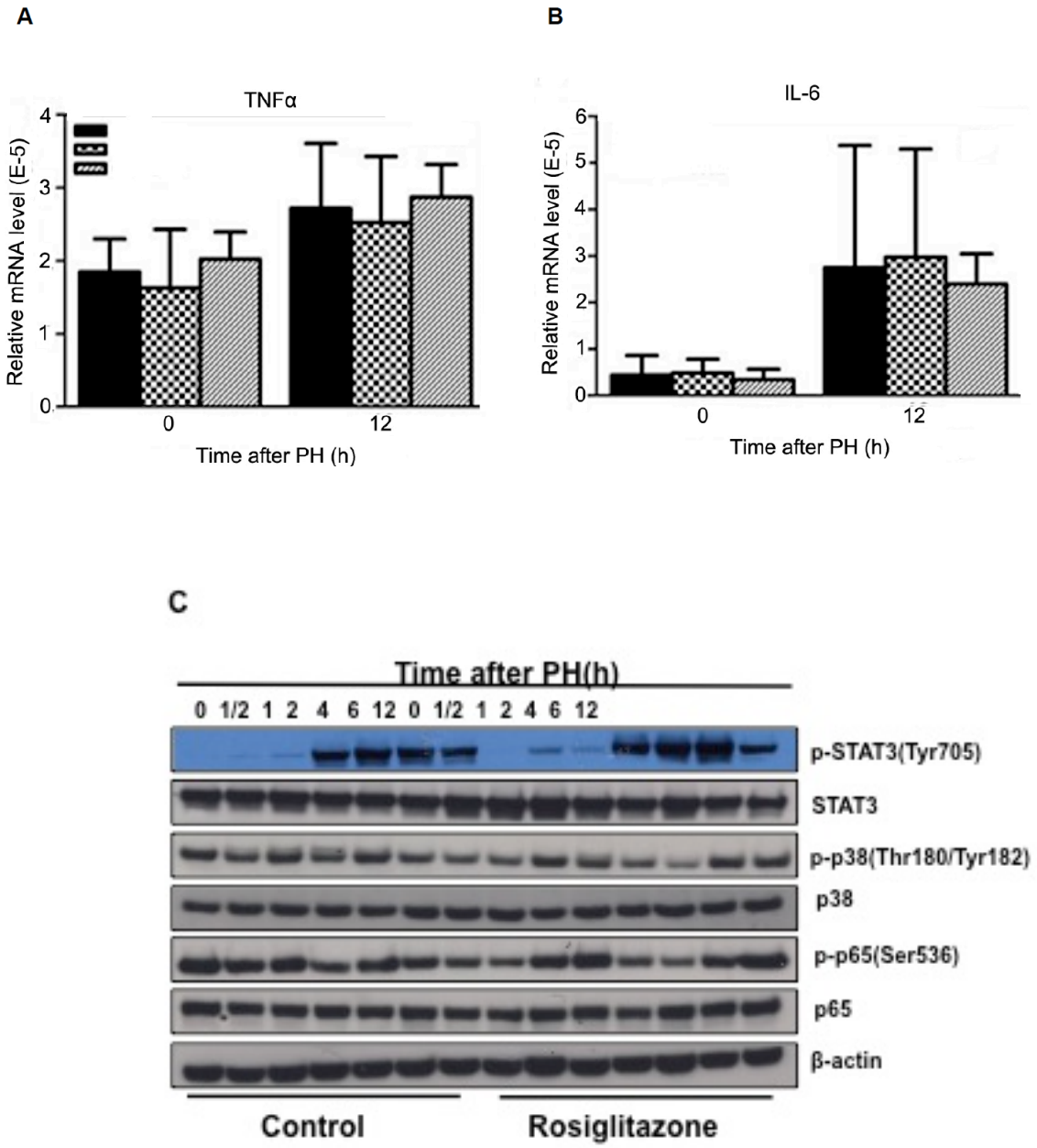


Figure 7. Effect of PPARγ on initiation of mouse liver regeneration.

A,B) Real-time PCR analysis of TNFα&IL6 mRNA exprssion at 0, 12h timepoint in control, rosiglitazone-treated, GW9662-treated groups. **C, D)** Western-blot analysis of STAT3, p38 MAP kinase, p65 activation **during early stage** of liver regeneration in rosiglitazone-treated and control mice.

4.3.2 PPARγ inhibits the activation of the HGF/c-Met/ERK1/2 signaling pathway

HGF is considered an extremely important growth factor as it promotes hepatocyte proliferation following PH (*Ishii T, et al., 1995*). To gain further insight into the underlying mechanism, we next investigated whether altered expression of PPARγ regulates the HGF/c-Met/ERK1/2 signaling pathway after PH. Following PH, HGF and HGF-induced phosphorylation of c-Met were dramatically increased over 12 h and maintained until 3 days after PH in each group of mice. As compared with control mice, the level of HGF activity was significantly lower in rosiglitazone-treated mice and subsequently resulted in lower activation of c-Met (Tyr1234/1235). In contrast, we observed slightly increased HGF activity in GW9662-treated mice and followed by remarkably increased phosphorylation of c-Met. In addition, the phosphorylation of ERK on Tyr202/204 was also decreased after PPARγ was activated by rosiglitazone (Figure 8). These results demonstrate that ligand activation of PPARγ down-regulates a subset of HGF-induced signaling events that inhibit hepatocyte proliferation.

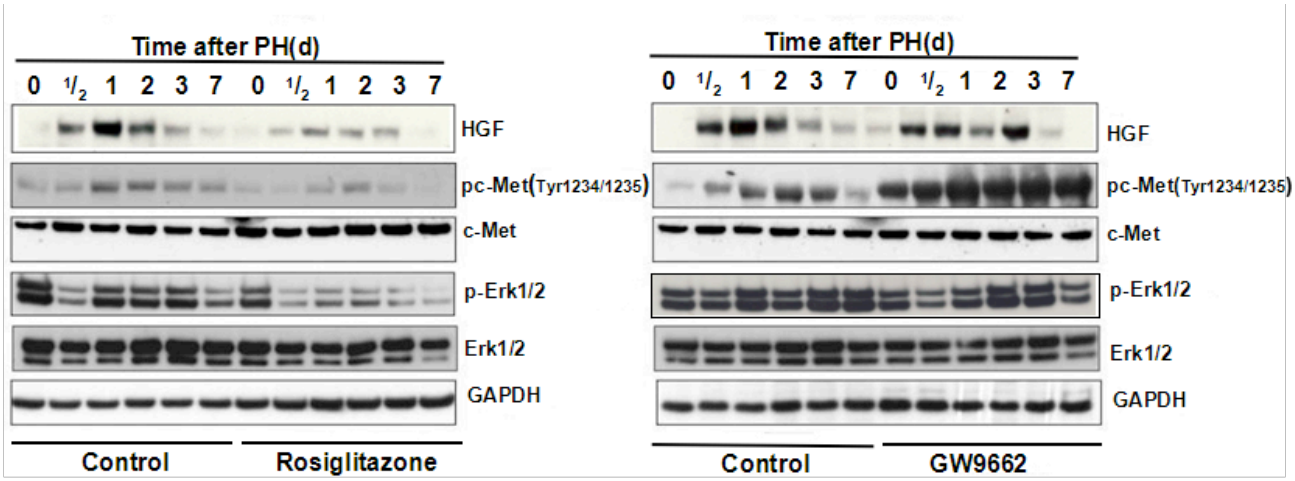
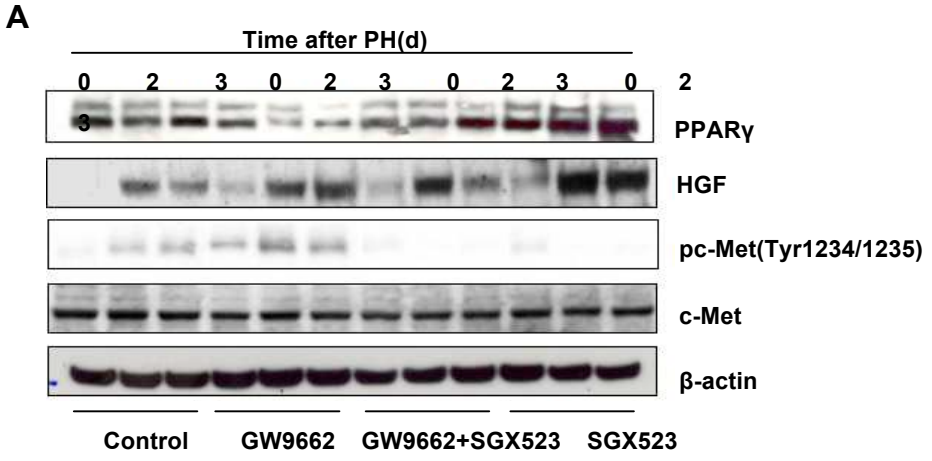


Figure 8. Protein expression of HGF/c-Met/ERK1/2 signaling pathway in regenerated livers.

Left panel: Hepatic expression of HGF/c-Met signaling pathway after PH in control vs. In rosigalitazone-treated mice. Right panel: Hepatic expression of HGF/c-Met signaling pathway after PH in control vs. in GW9662-treated mice. The immunoblots are representative of replicate analyses of 4 mice for each time point.

To further confirm that PPARγ inhibits liver regeneration via the HGF/c-Met/ERK1/2 signaling pathway, we investigated whether PPARγ antagonist treatment still augmented liver regeneration if we blocked the activation of c-Met using SGX523 by oral gavage. We observed that phosphorylation of c-Met was almost abolished in SGX523-treated and SGX523 plus GW9662-treated mice (Figure 9A). Significantly augmented liver regeneration (higher liver body ratio and percentage of Ki67 and PH3 positive hepatocytes) was observed in GW9662-treated mice compared to SGX523 plus GW9662-treated or SGX523-treated mice. In addition, a comparison of the liver body ratio and percentage of Ki67 and PH3 positive hepatocytes at 2 and 3 days post-PH showed no difference between the SGX523 plus GW9662-treated and SGX523-treated groups (Figure 9B, C, D). These findings demonstrate that blocking c-Met activation attenuates the effect of a PPARγ antagonist on promoting a subset of HGF-induced signaling pathways, which further validated that PPARγ controls liver regeneration by regulating HGF/c-Met signaling in partially hepatectomized mice.



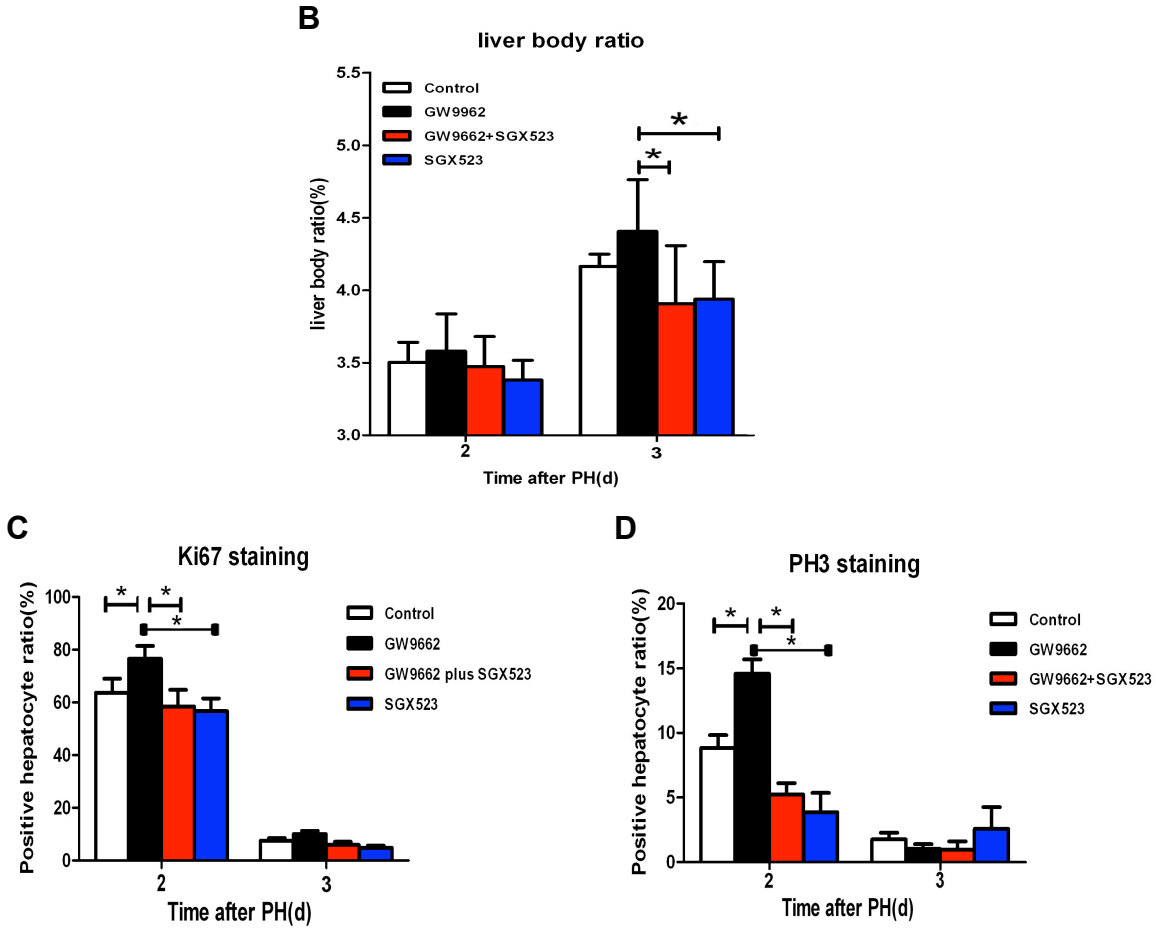
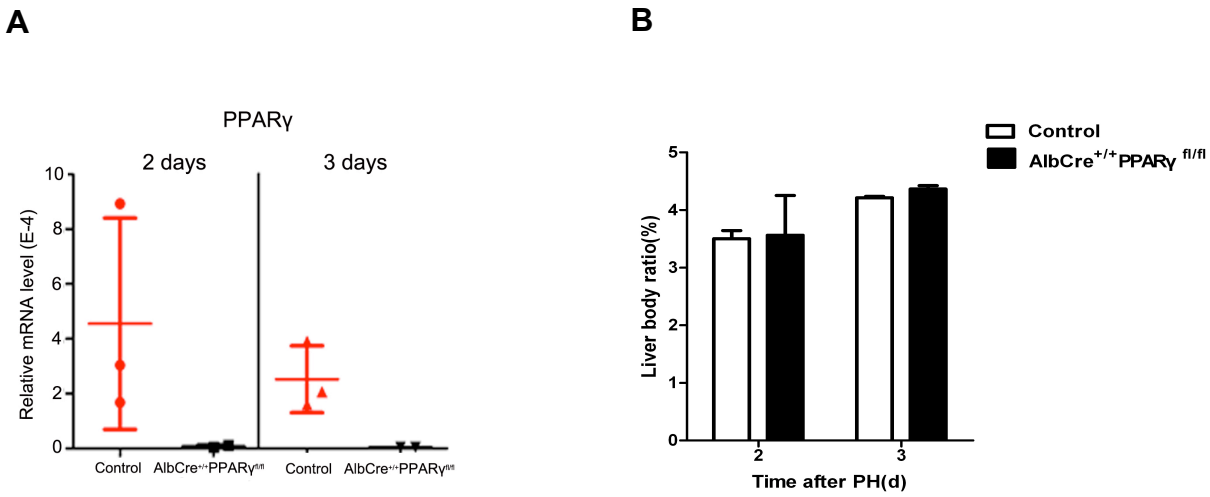


Figure 9. The influence of single or combinated apply of c-Met antagonist on hepatocyte proliferation. **A)** Liver to body weight ratio in control mice, GW9662-treated mice, SGX523-single-treated mice and GW9665-plus-SGX523-treated mice at 2 and 3 days after PH. **B, C)** IHC for hepatic Ki67 and PH3 after hepatectomy in four different groups. **D)** Protein expression of PPAR γ , HGF/c-met signaling pathway at 0, 2, 3 days time points after PH in 4 different groups. * p<0.05.

4.4 Regenerative response to PH is not accelerated in hepatocyte-specific PPARγ knock mice

The above results implied that inhibition of PPARγ in the liver leads to slightly accelerated liver regeneration, so we were curious as to whether the loss of PPARγ within hepatocytes would lead to enhanced liver recovery after PH. To further assess the functional role and regulation of PPARγ in liver regeneration and whether its effect is cell-type specific in hepatocytes, $AlbCre^{+/+}PPAR\gamma^{fl/fl}$ mice were subjected to PH and compared with control mice. Hepatocyte-specific deletion of PPARγ expression was confirmed using PCR (Figure 10A).

To our surprise, analysis of the liver body ratio showed that no significant difference was observed in AlbCre$^{+/+}$PPARγ$^{fl/fl}$ animals as compared to control mice at 2 days or 3 days post-PH, which corresponds to peak proliferation during liver regeneration (Figure 10B). We next investigated whether cell cycle progression was altered in AlbCre$^{+/+}$PPARγ$^{fl/fl}$ during the regenerative response. In both AlbCre$^{+/+}$PPARγ$^{fl/fl}$ mice and control mice, approximately 60% and 10% of hepatocytes, respectively, were positive of Ki67 and PH3 staining 2 days after PH. However, a significant difference between the groups was not found (Figure 10C, D, E, F). Consistent with the histological findings, western blot analysis showed comparable cyclin D1 expression in AlbCre$^{+/+}$PPARγ$^{fl/fl}$ mice and control mice 2 and 3 days post-PH (Figure 10G). These findings suggest that hepatocyte-specific loss of PPARγ neither compromises nor augments hepatocyte proliferation induced by PH. Furthermore, we may hypothesize that activation of PPARγ is especially critical in non-parenchymal cells to block the activation of growth factors (possibly HGF) and thus provides negative regulation of cell cycle progression in response to PH. Well-conducted studies are certainly needed to further validate this assumption.



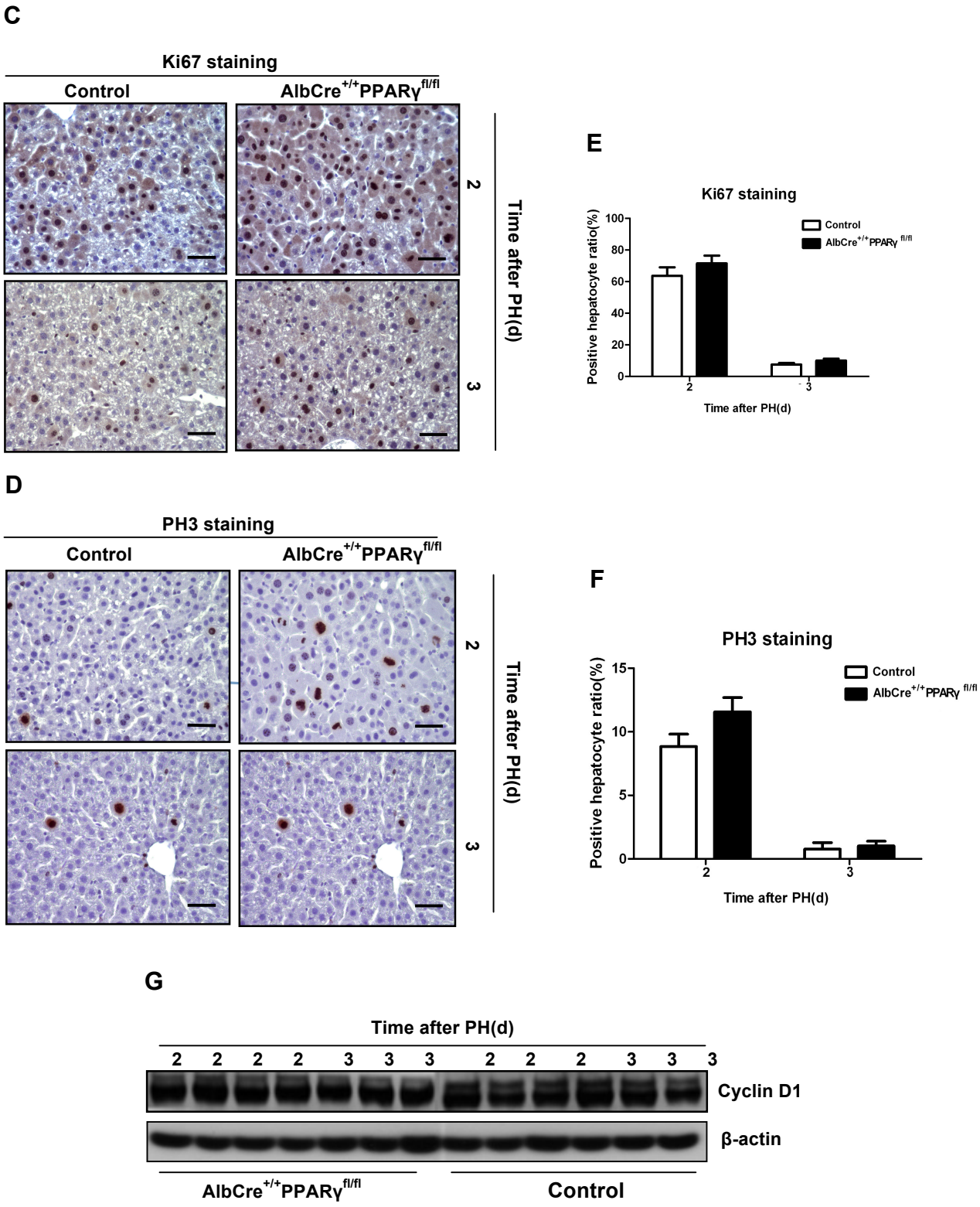


Figure 10. Regenerative response to PH is not accelerated in hepatocyte-specific PPARγ knock-out mice

A) qRT-PCR to assess the expression of PPARγ mRNA in AlbCre$^{+/+}$PPARγ$^{fl/fl}$ mice. **B)** Liver to body weight ratio in control mice, AlbCre$^{+/+}$PPARγ$^{fl/fl}$ at 2 and 3 days after PH. **C,D)** Micrographs of liver sections immunostained with Ki67 and PH3 antibodies from the two different groups after partial hepatectomy. Scale bar: 200 µm. **C, D)** Numbers of Ki67-positive hepatocytes and PH3-positive hepatocytes. **E)** Assessment of cyclin D1 protein expression in the regenerating liver.

5 DISCUSSION

5.1 PPARγ is a negative regulator of cell cycle progression and proliferation during liver regeneration

The study presented here was performed to investigate the role of PPARγ expression on hepatocellular proliferation during mouse liver regeneration induced by PH. The results show that after PPARγ was activated by rosiglitazone, regeneration was inhibited and the liver could not reach its original mass; the restoration of the liver was slightly accelerated after the expression of PPARγ was attenuated by GW9662. Consistent with previous studies, our results further confirm that PPARγ does inhibit mouse liver regeneration. Recently, Gazit et al. reported an accelerated regenerative response in liver-specific PPARγ null mice while augmented PPARγ expression in fatty liver led to an impaired regenerative response. In the present study, we inhibited PPARγ expression using GW9662, a specific antagonist of PPARγ, which is similar to global knockdown of PPARγ. We drew a similar conclusion since regeneration was in fact accelerated after PPARγ expression was attenuated by GW9662.

PPARγ is expressed in various tissue or cell types, including adipose tissue, hepatocytes, fibroblasts and epithelial cells (*Chawla A, et al., 1994; Tontonoz P, et al., 1994; Greene ME, et al., 1995; Braissant O, et al., 1996*). Our studies show that there is a strong expression in normal mouse liver tissue. Interestingly, PPARγ expression has a distinct distribution pattern that was altered during liver regeneration. After PH, PPARγ expression was significantly reduced during the early phase of regeneration when hepatocytes multiplied, and recovered gradually during the late phase of regeneration. This phenomenon indicates that PPARγ expression is homeostatic (self-regulating) during the process of regeneration, and it has to be down-regulated through some pathway to meet the requirements of hepatocyte proliferation or mitosis, then up-regulated again when proliferation has to be stopped. This finding from another point of view further confirmed that PPARγ does inhibit liver regeneration.

Studies have shown that ligand activation of PPARγ can induce growth inhibition through cell cycle arrest at the G1/S checkpoint in hepatic oval cells (*Cheng J, et al., 2004*), human pancreatic carcinoma cells (*Motomura W, et al., 2000*) or in vascular smooth muscle cells (*Wakino S, et al.,*

2000). PPARγ can also inhibit cell proliferation, likely by arresting the cell in the G2/M phase or inducing apoptosis in malignant or non-malignant tissue (*Yu J, et al., 2010; Yang FG, et al., 2005*). Our data show that the numbers of hepatocytes in the S and M phases were decreased and delayed after PPARγ was activated by rosiglitazone while the numbers were significantly increased after PPARγ was inhibited by GW9662. Furthermore, no obvious hepatocyte apoptosis was detected during the process of liver regeneration, either in the rosiglitazone group or in the GW9662 group. In addition, Gazit et al. found that accelerated induction of hepatic cyclin D1 expression after PH in liver-specific PPARγ null mice may account for the augmented regeneration observed in these animals. Our findings show that induction of hepatic cyclin D1 expression after PH was delayed 12 h after the activation of PPARγ by rosiglitazone. Taken together, these data indicate that PPARγ inhibits hepatocyte proliferation and delays cell cycle entry.

5.2 Activation of PPARγ does not suppress early cytokine signaling in liver regeneration

To address the underlying molecular mechanism of how PPARγ inhibits liver regeneration, we first investigated the early cytokine signaling pathways that are known to be important for the initiation of liver regeneration (*Taub R, 2004*). Indeed, TNFα and IL-6 were augmented during the first 12 h, and STAT3, p38 MAP kinase, p65 were activated over the first 6 h after hepatectomy both in rosiglitazone-treated mice and control mice; however, no significant differences were obtained between groups at any time point. These data imply that PPARγ does not result from the suppression of early cytokine signaling to inhibit mouse liver regeneration. This conclusion seems to conflict with published observations that rosiglitazone can suppress lipopolysaccharide (LPS)-stimulated TNFα production, and thus directly or indirectly regulate IL-6 secretion and activation of transcription factors (*Enomoto N, et al., 2005; Zingarelli B, et al., 2003*). However, this apparent contradiction can be reconciled by more recent reports: first, proinflammatory cytokine production during liver regeneration is independent of LPS signaling (*Campbell JS, et al., 2006*), and TNFα is also not necessary for normal liver regeneration since TNF knockout mice appear to regenerate normally. Second, although IL-6 accounts for almost 40% expression of the immediate early genes in the regenerating liver and IL-6 deficient mice exhibit impaired liver regeneration,

moderate IL-6 is functional well to activate downstream gene expression. Studies have also shown that IL-6 supplementation can suppress hepatocellular proliferation in wild-type mice subjected to PH, raising the possibility that the impaired regenerative response in rosiglitazone-treated animals may even be the result of increased IL-6. Furthermore, PPARγ directly or indirectly activates STAT3, p38 MAP kinase and NF-κB in a cell type-dependent manner. Here, we observed changes in transcriptional factors in the entire liver tissue, rather than in hepatocytes and non-parenchymal cells separately. This limitation may hide the effect of PPARγ on initial transcriptional factor activity. For now, the regulation of STAT3, p38 MAP kinase and NF-κB activity in different liver cell types during the regenerative response are still controversial; for example, the current concept suggests that activation of NF-κB is especially critical in non-parenchymal cells to produce cytokines (TNFα and IL-6) to adequately prime hepatocytes to proliferate after PH, while NF-κB within hepatocytes mainly has cytoprotective functions.

5.3 PPARγ negatively regulates the activation of HGF/c-Met during liver regeneration

Many studies have demonstrated that, in addition to cytokine-dependent pathways, several growth factors function to promote cellular replication during liver regeneration. HGF is one of the most important hepatocyte mitogens. It has been demonstrated that HGF/c-Met signaling plays a critical role during liver regeneration and repair after liver injury (*Huh CG, et al., 2004*). HGF protein levels in plasma and c-Met activity are increased immediately after PH in rats (*Stolz DB, et al., 1999; Pediaditakis P, et al., 2001; Michalopoulos GK, 2007*). HGF overexpression or exogenous HGF have been shown to induce hepatocyte proliferation and accelerate the process of liver regeneration following PH in mice (*Bell A, et al., 1999; Sakata H, et al., 1996; Shiota G, et al., 1998; Patijn GA, et al., 1998*). When HGF and c-Met re silenced in vivo by RNA interference, liver regeneration is impaired, and the expression pattern in many cell cycle- and apoptosis-related genes is abnormal (*Paranjpe S, et al., 2007*). To date, no sufficient studies have previously analyzed the relationship between PPARγ and the HGF/c-Met signaling pathway, particularly in liver regeneration. A previous study has shown that PPARγ activation by telmisartan has a renal protective action in mice with renal atrophy and fibrosis, and the effect of telmisartan is associated with significantly increased

renal HGF expression that is attenuated by GW9662, suggesting that HGF is one of the positively-regulated downstream effectors of PPARγ (*Kusunoki H, et al., 2012*). In contrast, our findings show that hepatic HGF as well as phosphorylated c-Met protein levels were significantly down-regulated after rosiglitazone treatment but up-regulated when PPARγ was inhibited by GW9662. Although the reason for the differences in our results and those of the previous study are uncertain, these conflicting results suggest that PPARγ may play different roles in different tissues or organs or pathophysiological processes.

Various studies have determined that PPARγ is one of the downstream effectors of ERK1/2, and it has been shown that activation of the ERK1/2 cascade can phosphorylate and thereby inhibit PPARγ activity (*Diradourian C, et al., 2005; Adams M, et al., 1997; Hu E, et al., 1996*). In addition, PPARγ phosphorylation by activation of the ERK1/2 cascade is assumed to be more prone to ubiquitination and subsequent degradation by the proteasome (Genini D, et al., 1996; Burns KA, et al., 2007; Hosooka T, et al., 2008). In the present study, our observations revealed a remarkable phenotype in that hepatic phosphorylated ERK1/2 protein levels were significantly down-regulated after PPARγ was activated by rosiglitazone. This interesting finding not only indicates that PPARγ inhibits mouse liver regeneration after PH via HGF/c-met/ERK1/2 pathways but also provided new evidence that PPARγ can also negatively regulate the phosphorylation of ERK1/2. It is worth performing further investigations concerning the interaction between PPARγ and the ERK1/2 cascade. In addition, our further experiments revealed that the HGF/c-Met signaling pathway was blocked by SGX523, and the accelerated liver regeneration by GW9662 was attenuated. These results further confirm that PPARγ inhibits mouse liver regeneration by inhibition of the HGF/c-met/ERK1/2 pathway.

In summary, our results support the concept that PPARγ inhibits liver growth and hepatocellular proliferation by inhibiting the HGF/c-met/ERK1/2 signaling pathway. These findings provide additional new insights into the role of PPARγ in efficient liver regeneration in response to the loss of liver mass and toxic liver injury.

5.4 Effects of PPARγ action are segmented in different cell and tissue types

In our study, hepatocyte-specific deletion of PPARγ showed no influence on hepatocyte proliferation induced by PH, raising the possibility that, during liver regeneration, ligand-mediated activation of PPARγ mainly inhibits cytokines and growth factors secretion in non-parenchymal cells, including Kupffer cells, hepatic stellate cells, and epithelial cells, to play its negative role. This inference is consistent with previous observations that suggest a multifaceted and tissue-specific effect of PPARγ.

In adipose tissue, PPARγ up-regulates genes involved in glucose uptake and also controls the expression of adipocyte-secreted factors, thus leading to insulin-sensitizing effects. Experiments with tissue-specific knockouts of PPARγ have been crucial in helping to dissect the relative contributions of PPARγ activity to insulin sensitization in different tissues. Mice with adipose-specific ablation of PPARγ show insulin resistance in adipose tissue and liver but not in skeletal muscle (*He W, et al., 2003*). In pancreatic beta cells, PPARγ induces the expression of key genes involved in glucose-stimulated insulin secretion (GSIS), although results from in vitro studies have shown that its ligands enhanced GSIS in insulin-resistant rodents and humans (*Kim HI, et al., 2002; Kim HI, et al., 2000*), conflicting with results from in vivo studies showing that the loss of PPARγ in the whole pancreas results in hyperglycemia with impaired GSIS (*Gupta D, et al., 2008; Rosen ED, et al., 2003*). PPARγ also has an important role in various immune cells, with most studies focusing on its role in antigen-presenting myeloid dendritic cells and macrophages (*Szanto A, et al., 2003*). In dendritic cells, PPARγ regulates lipid metabolism and transport as well as various other processes, including antigen uptake, maturation, activation, migration, cytokine production and antigen presentation (*Széles L, et al., 2007*). Macrophage PPARγ is implicated in anti-inflammation and lipid metabolism (*Wahli W, et al., 2012; Lee CH, et al., 2002; Nagy L, et al., 2008*). Notably, the action of PPARγ in different tumor cells also differs depending tissue source. In tumorigenesis, PPARγ may act as a potential link between energy balance, cellular metabolism and cancer pathogenesis, but its exact roles during carcinogenesis and tumor cell growth are still unclear.

Taken together, the results of these studies support the concept that effects of PPARγ are segmented to different cell and tissue types. We now better understand the possibility that activation

of PPARγ is especially critical in non-parenchymal cells to negatively regulate cytokines and growth factors to adequately prime hepatocytes to proliferate after PH, although PPARγ is mainly expressed in hepatocytes.

5.5 Translating insights into PPARγ to the clinic

Although thiazolidinediones (TZDs), as ligands of PPARγ, clearly have potent antidiabetic effects and are also used for treating patients suffering from liver fibrosis, non-alcoholic steatohepatitis and cancer due to their antifibrotic and proapoptotic effects, it is now apparent that they are accompanied by a myriad of unwanted side effects; as suggested in our study, administration of synthetic PPARγ ligands may suppress liver regeneration following hepatectomy. This finding provides support for current clinical practice in which these drugs should be restrainedly considered in patients with known or suspected liver injury. However, the results of this preliminary study should be interpreted cautiously and need further studies before clinical application.

Furthermore, our study provides compensatory evidence to support the current concept that the positive and negative effects of PPARγ action are segmented to different cell and tissue types, and thus tissue-targeted TZDs could be a future therapeutic strategy. Tissue-specific knockouts of PPARγ in mice can be employed to reveal complicated effects and the underlying mechanism of PPARγ on a single cell or tissue type. Basing on these experiments, new classes of highly targeted and effective drugs that preserve the strong positive efficacy of TZDs yet eliminate many of the unwanted side effects are possible. This translating insight is consistent with the concept of a “selective PPARγ modulator”, which is based on the idea that structurally distinct PPARγ ligands will result in unique receptor-ligand conformations with signature affinities for different co-regulators, thereby allowing discrete gene activation profiles within different cells and tissue types (*Takada I, et al., 2010*). Fortunately, a promising future of PPARγ-targeted therapeutics can be predictable to exert the “good” potent effects of PPARγ and simultaneously reduce or eliminate the “bad” associated side effects.

5.6 Study limitations and future directions

PPARγ has been demonstrated to play important and distinct regulatory roles in lipid metabolism, but much less is known about the pathways stimulated by PPARγ as well as mediators of the cell cycle in liver regeneration or hepatocellular carcinoma. Therefore, further studies should be performed to fully understand the functions, regulation mechanisms and signaling pathways associated with PPARγ in the normal liver, in order to better conceptualize the high levels PPARγ in hepatocytes. The interaction between PPARγ, cytokines and growth factors during liver regeneration are well worth investigating to better understand the correlation between the hepatocytes and non-parenchymal cells and the involved signaling networks. Unfortunately, in our study, we only found HGF to be regulated by PPARγ in regenerated liver. The correlation between PPARγ and HGF and the potential mechanism should be further observed and investigated using hepatocyte-specific deletion ($AlbCre^{+/+}PPAR\gamma^{fl/fl}$ mice) and liver-specific knockout mice ($MxCre^{+/+}PPAR\gamma^{fl/fl}$ mice) in the PH model. Furthermore, using a PPARγ transgenic mouse model, further investigating the effect of PPARγ on cell cycle control under different conditions, such as chemical-induced acute liver inflammation, liver necrosis and tumorigenesis in the liver can be conducted. Finally, since PPARγ has been found to influence inflammation and can regulate the immune response, it is also important to study the effect of PPARγ on the recruitment and proliferation of immunocytes in the liver induced by liver injury, which was beyond the scope of our study. This would provide more evidence for constructing the general picture of PPARγ during embryogenesis, organogenesis and tumorigenesis.

6 SUMMARY

Nuclear transcription factor peroxisome proliferator-activated receptor γ (PPARγ) is essential for liver homeostasis. Ligand activation of PPARγ was demonstrated to inhibit hepatocyte proliferation after 2/3 PH. However, the underlying molecular mechanisms remain unclear. In the present work, we modulated PPARγ activity in the liver using specific drugs and hepatocyte-specific PPARγ knockout mice and analyzed the role of PPARγ in the context of liver regeneration following PH.

Eight- to ten-week-old female C57BL/6J mice were treated with the PPARγ agonist rosiglitazone, the PPARγ antagonist GW9662 alone or in combination with the c-met inhibitor SGX523 and then subjected to 2/3 PH. The results show that PH in PPARγ agonist treated mice showed delayed liver regeneration, but regeneration was slightly accelerated in the PPARγ antagonist group. However, the triggered activation of initial cytokines and the subsequent cytokine-induced activation of STAT3, NF-κB, and p38 MAP kinase were not influenced by ligand activation of PPARγ. However, we did find that HGF, phosphorylated c-Met and phosphorylated ERK1/2 protein levels were significantly down-regulated after PPARγ agonist treatment with the opposite tendency after PPARγ antagonist treatment. Additionally, inhibition of c-met abrogated the augmenting effect of GW9662 on liver regeneration. These results suggest that PPARγ inhibits liver growth and hepatocellular proliferation by inhibiting the HGF/c-met/ERK1/2 signaling pathway.

Evidence from AlbCre$^{+/+}$PPARγ$^{fl/fl}$ animals showed that specific hepatocyte loss of PPARγ neither compromises nor augments hepatocyte proliferation induced by PH at the 2 and 3 day time points. These results imply that activation of PPARγ may be especially critical in non-parenchymal cells to block the activation of growth factors (possibly HGF) and thus provides negative regulation of cell cycle progression in response to PH. Well-conducted studies are certainly needed to further validate this assumption.

Altogether, our data establish that PPARγ activation attenuates the HGF/c-met/ERK1/2 pathway and thus negative regulation of HGF signaling could be an important mechanism underlying the inhibitory role of PPARγ on liver regeneration.

7 REFERENCE

1. Adams M, Reginato MJ, Shao D, Lazar MA, Chatterjee VK. Transcriptional activation by peroxisome proliferator-activated receptor gamma is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. J Biol Chem 1997; 272(8): 5128-5132.

2. Akerman P, Cote P, Yang SQ, McClain C, Nelson S, Bagby GJ, Diehl AM. Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. Am J Physiol 1992;263(4 Pt 1): G579 -585.

3. Albrecht JH, Hoffman JS, Kren BT, Steer CJ. Cyclin and cyclin-dependent kinase 1 mRNA expression in models of regenerating liver and human liver diseases. Am J Physiol 1993; 265(5 Pt 1): G857-864.

4. Anderson SP, Yoon L, Richard EB, Dunn CS, Cattley RC, Corton JC. Delayed liver regeneration in peroxisome proliferator-activated receptor-alpha-null mice. Hepatology 2002; 36(3): 544-554.

5. Apte U, Gkretsi V, Bowen WC, Mars WM, Luo JH, Donthamsetty S, Orr A, Monga SP, Wu C, Michalopoulos GK. Enhanced liver regeneration following changes induced by hepatocyte-specific genetic ablation of integrin-linked kinase. Hepatology 2009; 50(3): 844-851.

6. Attisano L, Wrana JL. Signal transduction by the TGF-beta superfamily. Science 2002; 296(5573): 1646-1647.

7. Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, Ellis E, Strom S, Kay MA, Finegold M, Grome M. Robust expansion of human hepatocytes in Fah-/-/Rag2-/-/Il2rg-/- mice. Nat Biotechnol 2007; 25(8): 903–910.

8. Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, Koder A, Evans RM. PPARγ is required for placental, cardiac, and adipose tissue development. Mol Cell 1999; 4(4): 585–595.

9. Behrens A, Sibilia M, David JP, Möhle-Steinlein U, Tronche F, Schütz G, Wagner EF. Impaired postnatal hepatocyte proliferation and liver regeneration in mice lacking c-jun in the liver. EMBO J 2002; 21(7): 1782-1790.

10. Bell A, Chen Q, DeFrances MC, Michalopoulos GK, Zarnegar R. The five amino acid-deleted isoform of hepatocyte growth factor promotes carcinogenesis in transgenic mice. Oncogene 1999; 18(4): 887-895.

11. Bishop-Bailey D, Hla T. Endothelial cell apoptosis induced by the peroxisome proliferator-activated receptor (PPAR) ligand 15-deoxyDelta12, 14-prostaglandin J2. J Biol Chem 1999: 274(24): 17042 -17048.

12. Bor MV, Shi Y, Sorensen BS, Wen JG, Frokiaer J, Djurhuus JC, Nexo E. Increased TGF-alpha and EGF Receptor mRNA expression in response to neonatal unilateral partial ureter obstruction in rats. Nephron Exp

Nephrol 2006; 104(2): e76- 82.

13. Borowiak M, Garratt AN, Wustefeld T, Strehle M, Trautwein C, Birchmeier C. Met provides essential signals for liver regeneration. Proc Natl Acad Sci USA 2004; 101(29): 10608-10613.

14. Borude P, Edwards G, Walesky C, Li F, Ma X, Kong B, Guo GL, Apte U. Hepatocyte-specific deletion of farnesoid X receptor delays but does not inhibit liver regeneration after partial hepatectomy in mice. Hepatology 2012; 56(6): 2344-2352.

15. Braissant O, Foufelle F, Scotto C, Dauc M, Wahli W. Diferential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta,and -gamma in the adult rat. Endocrinology 1996; 137(1): 354–366.

16. Burgermeister E, Chuderland D, Hanoch T, Meyer M, Liscovitch M, Seger R. Interaction with MEK causes nuclear export and downregulation of peroxisome proliferator-activated receptor. Mol Cell Biol 2007;27(3): 803–817.

17. Burgermeister E, Seger R. MAPK kinases as nucleo-cytoplasmic shuttles for PPAR. Cell Cycle 2007; 6(13): 1539-1548.

18. Burgess HA, Daugherty LE, Thatcher TH, Lakatos HF, Ray DM, RedonnetM, Phioos RP, Sime PJ.. PPARgamma agonists inhibit TGF-beta induced pulmonary myofibroblast differentiation and collagen production: implications for therapy of lung fibrosis. Am J Physiol Lung Cell Mol Physiol 2005; 288(6): L1146–1153.

19. Burns KA, Vanden Heuvel JP. Modulation of PPAR activity via phosphorylation. Biochim Biophys Acta 2007; 1771(8): 952-960.

20. Camp HS, Tafuri SR. Regulation of peroxisome proliferator-activated receptor gamma activity by mitogen-activated protein kinase. J Biol Chem 1997 ; 272(16):10811-10816.

21. Campbell JS, Riehle KJ, Brooling JT, Bauer RL, Mitchell C, Fausto N. Pro-inflammatory cytokine production in liver regeneration is Myd88-dependent, but independent of Cd14, Tlr2, and Tlr4. J Immunol 2006; 176(4): 2522-2528.

22. Castrillo A, Diaz-Guerra MJ, Hortelano S, Martin-Sanz P, Bosca P. Inhibition of IkappaB kinase and IkappaB phosphorylation by 15-deoxy-Delta(12,14)-prostaglandin J(2) in activated murine macrophages. Mol Cell Biol 2000; 20(5): 1692–1698.

23. Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RR, Xu HE, Turk J, Semenkovich CF. Identification of a physiologically relevant endogenous ligand for PPARalpha in liver. Cell 2009; 138(3): 476-488.

24. Chang TH, Szabo E. Induction of differentiation and apoptosis by ligands of peroxisome proliferator-activated receptor gamma in non-small cell lung cancer. Cancer Res 2000; 60(4): 1129-1138.

25. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, Liao D, Nagy L, Edwards PA, Curtiss LK, Evans RM, Tontonoz P. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 2001; 7(1): 161-171.

26. Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA. Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. Endocrinology 1994; 135(2): 798-800.

27. Chen H, Sun Y, Dong R, Yang S, Pan C, Xiang D, Miao M, Jiao B. Mir-34a is up-regulated during liver regeneration in rats and is associated with the suppression of hepatocyte proliferation. PLoS One 2011; 6(5): e202-238.

28. Cheng J, Nakamura H, Imanishi H, Liu W, Morisaki T, Sugiyama T, Hada T. Peroxisome proliferator-activated receptor gamma ligands, 15-deoxy-Delta12,14-prostaglandin J2, and ciglitazone, induce growth inhibition and cell cycle arrest in hepatic oval cells. Biochem Biophys Res Commun 2004; 322(2) :458-464.

29. Choy L, Derynck R. Transforming growth factor-beta inhibits adipocyte differentiation by Smad3 interacting with CCAAT/enhancer-binding protein (C/EBP) and repressing C/EBP transactivation function. J Biol Chem. 2003; 278(11): 9609-9619.

30. Chuang CC, Yang RS, Tsai KS, Ho FM, Liu SH. Hyperglycemia enhances adipogenic induction of lipid accumulation: involvement of extracellular signal-regulated protein kinase 1/2, phosphoinositide 3-kinase/Akt, and peroxisome proliferator-activated receptor gamma signaling. Endocrinology 2007;148(9):4267–4275.

31. Coll T, Jové M, Rodríguez-Calvo R, Eyre E, Palomer X, Sánchez RM, Merlos M, Laguna JC, Vázquez-Carrera M. Palmitate-mediated downregulation of peroxisome proliferator-activated receptor-gamma coactivator 1alpha in skeletal muscle cells involves MEK1/2 and nuclear factor-kappaB activation. Diabetes 2006; 55(10): 2779-2787.

32. Court FG, Wemyss-Holden SA., Dennison AR, Maddern GJ. The mystery of liver regeneration. Br J Surg 2002; 89(9): 1089-1095.

33. Coutant A, Rescan C,Gilot D, Loyer P, Guguen-Guillouzo C, Baffet G. PI3K-FRAP/mTOR pathway is critical for hepatocyte proliferation whereas MEK/ERK supports both proliferation and survival. Hepatology 2002; 36(5): 1079-1088.

34. Cressman DE, Diamond RH, Taub R. Rapid activation of the Stat3 transcription complex in liver regeneration. Hepatology 1995; 21(5): 1443--1449.

35. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 1996; 274(5291): 1379-1383.

36. Cressman DE, Greenbaum LE, Haber BA, Taub R. Rapid activation of post-hepatectomy factor/nuclear factor kappa B in hepatocytes, a primary response in the regenerating liver. J Biol Chem, 1994; 269(48): 30429-30435.

37. Croker BA, Krebs DL, Zhang JG, Wormald S, Willson TA, Stanley EG. SOCS3 negatively regulates IL-6 signaling in vivo. Nat Immunol 2003; 4(6): 540-545.

38. Crumm S, Cofan M, Juskeviciute E, Hoek JB. Adenine nucleotide changes in the remnant liver: an early signal for regeneration after partial hepatectomy. Hepatology 2008; 48(3):898-908.

39. Decker K, Keppler D. Galactosamine induced liver injury. Prog Liver Dis 1972; 4:183-199.

40. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 2003; 425(6958): 577-584.

41. Desbois-Mouthon C, Wendum D, Cadoret A, Rey C, Leneuve P, Blaise A, Housset C, Tronche F, Le Bouc Y, Holzenberger M.I. Hepatocyte proliferation during liver regeneration is impaired in mice with liver-specific IGF-1R knockout..FASEB J 2006; 20(6): 773-5.

42. Dierssen U, Beraza N, Lutz HH, Liedtke, Ernst M, Wasmuth HE, Trautwein C. Molecular dissection of gp130-dependent pathways in hepatocytes during liver regeneration. J Biol Chem 2008; 283(15): 9886-9895.

43. Diradourian C, Girard J, Pegorier JP. Phosphorylation of PPARs: from molecular characterization to physiological relevance. Biochimie 2005; 87(1): 33-38.

44. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W. Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell 1992; 68(5): 879-887.

45. Enomoto N, Takei Y, Yamashima S, Ikejima K, Kitamura T, Sato N. Protective effect of pioglitazone against

endotoxin-induced liver injury through prevention of Kupffer cell sensitization. Alcohol Clin Exp Res 2005; 29(12 Suppl): 216S–219S.

46. Espeillac C, Mitchell C, Celton-Morizur S, Chauvin C, Koka V, Gillet C, Albrecht JH, Desdouets C, Pende M. S6 kinase 1 is required for rapamycin-sensitive liver proliferation after mouse hepatectomy. J Clin Invest 2011; 121(7): 2821-2832.

47. Fajas L, Fruchart JC, Auwerx J. PPARgmma3 mRNA:adistinct PPAR mRNA subtype transcribed from an independent promoter. FEBS Letters 1998; 438(1-2): 55-60.

48. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology 2006; 43(2 Suppl 1): S45-53.

49. Fausto N, Riehle KJ. Mechanisms of liver regeneration and their clinical implications. J Hepatobiliary Pancreat Surg 2005; 12(3): 181--189.

50. Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. Hepatology 2004; 39(6): 1477-1487.

51. FitzGerald MJ, Webber EM, Donovan JR, Fausto N. Rapid DNA binding by nuclear factor kappa B in hepatocytes at the start of liver regeneration. Cell Growth Differ 1995; 6(4): 417-427.

52. Forman BM, Chen J, Evans RM. The peroxisome proliferator-activated receptors: ligands and activators. Ann NY Acad Sci 1996; 804: 266-275.

53. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-deoxy-Δ12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR. Cell 1995; 83(5): 803-812.

54. Freeman TL, Ngo HQ, Mailliard ME. Inhibition of system A amino acid transport and hepatocyte proliferation following partial hepatectomy in the rat. Hepatology 1999; 30(2): 437-444.

55. Fujita J, Marino MW, Wada H, Jungbluth AA, Mackrell PJ, Rivadeneira DE, et al. Effect of TNF gene depletion on liver regeneration after partial hepatectomy in mice. Surgery 2001; 129(1): 48-54.

56. Gaub J, Iversen J. Rat liver regeneration after 90% partial hepatectomy. Hepatology 1984; 4(5): 902-904.

57. Gazit V, Huang J, Weymann A, Rudnick DA. Analysis of the role of hepatic PPARgamma expression during mouse liver regeneration. Hepatology 2012; 56(4): 1489-1498.

58. Gazit V, Weymann A, Hartman E, Finck BN, Hruz PW, Tzekov A, Rudnick DA. Liver regeneration is impaired in lipodystrophic fatty liver dystrophy mice. Hepatology 2010; 52(6): 2109-2117.

59. Gebhardt R. Altered acinar distribution of glutamine synthetase and different growth response of cultured enzyme-positive and -negative hepatocytes after partial hepatectomy. Cancer Res 1990; 50(14): 4407-4410.

60. Genini D, Catapano CV. Control of peroxisome proliferator-activated receptor fate by the ubiquitinproteasome system. J Recept Signal Transduct Res 2006; 26(5-6): 679-692.

61. Goggin MM, Nelsen CJ, Kimball SR, Jefferson LS, Morley SJ, Albrecht JH. Rapamycin-sensitive induction of eukaryotic initiation factor 4F in regenerating mouse liver. Hepatology 2004; 40(3): 537-544.

62. Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K, Hsieh L, Greene G, Nimer SD.. Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. Gene Expr 1995; 4(4-5): 281-299.

63. Gupta D, Jetton TL, Mortensen RM, Duan SZ, Peshavaria M, Leahy JL. In vivo and in vitro studies of a functional peroxisome proliferator-activated receptor γ response element in the mouse pdx-1 promoter. J Biol Chem. 2008; 283(47): 32462–32470.

64. Han C, Demetris AJ, Liu Y, Shelhamer JH, Wu T. Transforming growth factor-beta (TGF-beta) activates cytosolic phospholipase A2alpha (cPLA2alpha)-mediated prostaglandin E2 (PGE)2/EP1 and peroxisome proliferator-activated receptor-gamma (PPAR-gamma)/Smad signaling pathways in human liver cancer cells. A novel mechanism for subversion of TGF-beta-induced mitoinhibition. J Biol Chem. 2004 22; 279(43): 44344-44354.

65. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev 2004;18 (16):1926-1945

66. Hayashi H, Nagaki M, Imose M, Osawa Y, Kimura K, Takai S, Imao M, Naiki T, Kato T, Moriwaki H.. Normal liver regeneration and liver cell apoptosis after partial hepatectomy in tumor necrosis factor-alpha-deficient mice. Liver Int 2005; 25(1): 162-170.

67. Hayashi H, Sakai K, Baba H, Sakai T. Thrombospondin-1 is a novel negative regulator of liver regeneration after partial hepatectomy through transforming growth factor-beta1 activation in mice. Hepatology 2012; 55(5): 1562-1573.

68. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM. Adipose-specific

peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle. Proc Natl Acad Sci USA 2003; 100(26): 15712-15717.

69. Heaney AP, Fernando M, Melmed S. PPAR-gamma receptor ligands: novel therapy for pituitary adenomas. J Clin Invest 2003; 111(9): 1381-1388.

70. Higgins GM, Anderson RM. Experimental pathology of the liver: restoration of the liver of the white rat following partial surgical removal. Arch Pathol 1931; 12:186–202.

71. Holecek M. Nutritional modulation of liver regeneration by carbohydrates, lipids, and amino acids: a review. Nutrition 1999; 15(10): 784-788.

72. Hosooka T, Noguchi T, Kotani K, Nakamura T, Sakaue H, Inoue H, Ogawa W, Tobimatsu K, Takazawa K, Sakai M, Matsuki Y, Hiramatsu R, Yasuda T, Lazar MA, Yamanashi Y, Kasuga M.. Dok1 mediates high-fat diet-induced adipocyte hypertrophy and obesity through modulation of PPAR-gamma phosphorylation. Nat Med 2008; 14(2): 188-193.

73. Hu E, Kim JB, Sarraf P, Spiegelman BM. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma. Science 1996; 274(5295): 2100-2103.

74. Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, Kelly C, Witztum JL, Funk CD, Conrad D, Glass CK. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. Nature 1999; 400(6742); 378-382.

75. Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, Dong B, Huang X, Moore DD. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. Science 2006; 312(577): 233-236.

76. 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 US A 2004; 101(13): 4477-4482.

77. Ichikawa T, Zhang YQ, Kogure K, Hasegawa Y, Takagi H, Mori M, Kojima I. Transforming growth factor beta and activin tonically inhibit DNA synthesis in the rat liver. Hepatology 2001; 34(5): 918-925.

78. Imai T, Takakuwa R, Marchand S, Dentz E, Bornert JM, Messaddeq N, Wendling O, Mark M, Desvergne B, Wahli W, Chambon P, Metzger D. Peroxisome proliferator-activated receptor γ is required in mature white and

brown adipocytes for their survival in the mouse. Proc Natl Acad Sci USA 2004; 101(13): 4543–4547.

79. Ishii T, Sato M, Sudo K, Suzuki M, Nakai H, Hishida T, Niwa T, Umezu K, Yuasa S. Hepatocyte growth factor stimulates liver regeneration and elevates blood protein level in normal and partially hepatectomized rats. J Biochem 1995; 117(5): 1105-1112.

80. Ji H, Wang H, Zhang F, Li X, Xiang L, Aiguo S. PPARgamma agonist pioglitazone inhibits microglia inflammation by blocking p38 mitogen-activated protein kinase signaling pathways. Inflamm Res 2010; 59(11): 921–929.

81. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; 391(6662): 82-86.

82. Jin J, Wang GL, Shi X, Darlington GJ, Timchenko NA. The ageassociated decline of glycogen synthase kinase 3beta plays a critical role in the inhibition of liver regeneration. Mol Cell Biol 2009; 29(14): 3867-3880.

83. Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature 2000; 405(6785): 421-424.

84. Kim HI, Cha JY, Kim SY, Kim JW, Roh KJ, Seong JK, Lee NT, Choi KY, Kim KS, Ahn YH. Peroxisomal proliferator-activated receptor-γ upregulates glucokinase gene expression in beta-cells. Diabetes. 2002; 51(3): 676–685.

85. Kim HI, Kim JW, Kim SH, Cha JY, Kim KS, Ahn YH. Identification and functional characterization of the peroxisomal proliferator response element in rat GLUT2 promoter. Diabetes. 2000; 49(9):1517–1524.

86. Kiso S, Kawata S, Tamura S, Inui Y, Yoshida Y, Sawai Y, Umeki S, Ito N, Yamada A, Miyagawa J, Higashiyama S, Iwawaki T, Saito M, Taniguchi N, Matsuzawa Y, Kohno K. Liver regeneration in heparin-binding EGF-like growth factor transgenic mice after partial hepatectomy. Gastroenterology 2003; 124(3): 701-707.

87. Knight B, Yeoh GC. TNF/LTalpha double knockout mice display abnormal inflammatory and regenerative responses to acute and chronic liver injury. Cell Tissue Res 2005; 319(1): 61-70.

88. Koga H, Sakisaka S, Harada M, Takagi T, Hanada S, Taniguchi E, Kawaguchi T, Sasatomi K, Kimura R, Hashimoto O, Ueno T, Yano H, Kojiro M, Sata M.. Involvement of p21(WAF1/Cip1), p27(Kip1), and p18(INK4c) in troglitazone-induced cell-cycle arrest in human hepatoma cell lines. Hepatology 2001; 33(5): 1087-1097.

89. Koniaris LG, McKillop IH, Schwartz SI, Zimmers TA. Liver regeneration. J Am Coll Surg 2003; 197(4): 634-59.

90. Kramer D, Shapiro R, Adler A, Bush E, Rondinone CM. Insulin-sensitizing efect of rosiglitazone (BRL-49653) by regulation of glucose transporters in muscle and fat of zucker rats. Metabolism 2001; 50(11): 1294-1300.

91. Kung J, Henry RR. Thiazolidinedione safety. Expert Opin Drug Saf 2012; 11(4): 565-579.

92. Kusunoki H, Taniyama Y, Azuma J, Iekushi K, Sanada F, Otsu R, Iwabayashi M, Okayama K, Rakugi H, Morishita R. Telmisartan exerts renoprotective actions via peroxisome proliferator-activated receptor-gamma/hepatocyte growth factor pathway independent of angiotensin II type 1 receptor blockade. Hypertension 2012; 59(2): 308-316.

93. Lee CH, Evans RM. Peroxisome proliferator-activated receptor-γ in macrophage lipid homeostasis. Trends Endocrinol Metab. 2002; 13(8): 331-335.

94. Lehmann K, Tschuor C, Rickenbacher A, Jang JH, Oberkofler CE, Tschopp O, Schultze SM, Raptis DA, Weber A, Graf R, Humar B, Clavien PA.. Liver failure after extended hepatectomy in mice is mediated by a p21-dependent barrier to liver regeneration. Gastroenterology 2012; 143(6):1609-1619.

95. Lehrke M, Lazar MA. The many faces of PPARgamma. Cell 2005;123(6): 993-999.

96. Lemire JM, Shiojiri N, Fausto N. Oval cell proliferation and the origin of small hepatocytes in liver injury induced by D-galactosamine. Am J Pathol 1991;139(3): 535–552.

97. Lesurte IM, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, Gachet C, Bader M, Clavien PA. Platelet-derived serotonin mediates liver regeneration. Science 2006; 312(5770):104–107.

98. Leu JI, Crissey MA, Leu JP, Ciliberto G, Taub R. Interleukin-6-induced STAT3 and AP-1 amplify hepatocyte nuclear factor 1-mediated transactivation of hepatic genes, an adaptive response to liver injury. Mol Cell Biol 2001; 21(2): 414--424.

99. Leu JI, Crissey MA, Taub R. Massive hepatic apoptosis associated with TGF-b1 activation after Fas ligand treatment of IGF binding protein-1–deficient mice. J Clin Invest 2003; 111(1): 129-139.

100. Levy DE, Lee CK. What does Stat3 do? J Clin Invest 2002; 109(9): 1143-1148.

101. 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; 277(32): 28411–28417.

102. Lin CW, Mars WM, Paranjpe S, Donthamsetty S, Bhave VS, Kang LI, Orr A, Bowen WC, Bell

AW, Michalopoulos GK. Hepatocyte proliferation and hepatomegaly induced by phenobarbital and 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene is suppressed in hepatocyte-targeted glypican 3 transgenic mice. Hepatology 2011; 54(2):620-630.

103. Maeda A, Horikoshi S, Gohda T, Tsuge T, Maeda K, Tomino Y. Pioglitazone attenuates TGF-beta(1)-induction of fibronectin synthesis and its splicing variant in human mesangial cells via activation of peroxisome proliferator-activated receptor (PPAR)gamma. Cell Biol Int 2005; 29(6): 422–428.

104. Malato Y, Sander LE, Liedtke C, Al-Masaoudi M, Tacke F, Trautwein C, Beraza N. Hepatocyte-specific inhibitor-of-kappaB-kinase deletion triggers the innate immune response and promotes earlier cell proliferation during liver regeneration. Hepatology 2008; 47(6): 2036-2050.

105. Matsuda Y, Matsumoto K, Yamada A, Ichida T, Asakura H, Komoriya Y, Nishiyama E, Nakamura T. Preventive and therapeutic effects in rats of hepatocyte growth factor infusion on liver fibrosis/cirrhosis. Hepatology 1997; 26(1): 81-89.

106. Menjo M, Ikeda K, Nakanishi M. Regulation of G1 cyclin-dependent kinases in liver regeneration. J Gastroenterol Hepatol 1998; 13 Suppl: S100–105.

107. Michalik L, Wahli W. Involvement of PPAR nuclear receptors in tissue injury and wound repair. J Clin Invest 2006; 116(3): 598-606.

108. Michalopoulos GK, Bowen WC, Mulè K, Stolz DB. Histological organization in hepatocyte organoid cultures. Am J Pathol 2001; 159(5): 1877-1887.

109. Michalopoulos GK, DeFrances MC. Liver regeneration. Science 1997; 276: 60-66.

110. Michalopoulos GK. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. Am J Pathol 2010;176(1):2–13.

111. Michalopoulos GK. Liver regeneration. J Cell Physiol 2007; 213(2): 286-300.

112. Mitchell C, Nivison M, Jackson LF, Fox R, Lee DC, Campbell JS, Fausto N. Heparin-binding epidermal growth factor-like growth factor links hepatocyte priming with cell cycle progression during liver regeneration. J Biol Chem 2005; 280(4):2562–2568..

113. Mitchell C, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. Nat

Protoc 2008; 3(7): 1167-1170.

114. Monga SP, Pediaditakis P, Mule K, Stolz DB, Michalopoulos GK. Changes in WNT/beta-catenin pathway during regulated growth in rat liver regeneration. Hepatology 2001; 33(5):1098-1109.

115. Moore KJ, Rosen ED, Fitzgerald ML, Randow F, Andersson LP, Altshuler D, Milstone DS, Mortensen RM, Spiegelman BM, Freeman MW. The role of PPAR-gamma in macrophage differentiation and cholesterol uptake. Nat Med 2001; 7(1): 41-47.

116. Motomura W, Okumura T, Takahashi N, Obara T, Kohgo Y. Activation of peroxisome proliferator-activated receptor gamma by troglitazone inhibits cell growth through the increase of p27KiP1 in human. Pancreatic carcinoma cells. Cancer Res 2000; 60(19): 5558-5564.

117. Motta PM. The three-dimensional fine structure of the liver as revealed by scanning electron microscopy. Int Rev Cytol Suppl 1977; (6): 347-399.

118. Motta PM. The three-dimensional microanatomy of the liver. Arch Histol Jpn 1984; 47(1): 1-30.

119. Moustakas A, Heldin CH. Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. Cancer Sci 2007; 98(10):1512-1520.

120. Myronovych A, Murata S, Chiba M, Matsuo R, Ikeda O, Watanabe M, Hisakura K, Nakano Y, Kohno K, Kawasaki T, Hashimoto I, Shibasaki Y, Yasue H,Ohkohchi N.. Role of platelets on liver regeneration after 90% hepatectomy in mice. J Hepatol 2008; 49(3): 363-372.

121. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage geneexpression through ligand activation of PPARγ. Cell 1998; 93(2): 229-240.

122. Nakamura K, Nonaka H, Saito H, Tanaka M, Miyajima A. Hepatocyte proliferation and tissue remodeling is impaired after liver injury in oncostatin M receptor knockout mice. Hepatology 2004; 39(3): 635--644.

123. Nanashima A, Omagari K, Sumida Y, Abo T, Fukuoha H, Takeshita H, Hidaka S, Tanaka K, Sawai T, Yasutake T, Nagayasu T, Mizuta Y, Ohba K, Ohnita K,Kadokawa Y. Evaluation of new prognostic staging systems (SLiDe score) for hepatocellular carcinoma patients who underwent hepatectomy. Hepatogastroenterolog 2009; 56(93):1137-1140.

124. Nelsen CJ, Rickheim DG, Tucker MM, Hansen LK, Albrecht JH. Evidence that cyclin D1 mediates both growth

and proliferation downstream of TOR in hepatocytes. J Biol Chem 2003; 278(6): 3656-3663.

125. Oe S, Lemmer ER, Conner EA, Factor VM, Levéen P, Larsson J, Karlsson S, Thorqeirsson SS. Intact signaling by transforming growth factor beta is not required for termination of liver regeneration in mice. Hepatology 2004; 40(5):1098-1105.

126. Paranjpe S, Bowen WC, Bell AW, Nejak-Bowen K, Luo JH, Michalopoulos GK. Cell cycle effects resulting from inhibition of hepatocyte growth factor and its receptor c-Met in regenerating rat livers by RNA interference. Hepatology 2007; 45(6): 1471-1477.

127. Park EJ, Park SY, Joe EH, Jou I. 15d-PGJ2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia. J Biol Chem 2003; 278(17): 14747-14752.

128. Park JY, Bae MA, Cheon HG, Kim SS, Hong JM, Kim TH, Lim J, Choi CH, Shin HI, Kim SY, Park EK. A novel PPARgamma agonist, KR62776, suppresses RANKL-induced osteoclast differentiation and activity by inhibiting MAP kinase pathways. Biochem Biophys Res Commun 2009; 378: 645–649.

129. Patijn GA, Lieber A, Schowalter DB, Schwall R, Kay MA. Hepatocyte growth factor induces hepatocyte proliferation in vivo and allows for efficient retroviral-mediated gene transfer in mice. Hepatology 1998; 28(3): 707-716.

130. Pediaditakis P, Lopez-Talavera JC, Petersen B, Monga SP, Michalopoulos GK. The processing and utilization of hepatocyte growth factor/scatter factor following partial hepatectomy in the rat. Hepatology 2001; 34(4 Pt 1): 688-693.

131. Poulsen L, Siersbaek M, Mandrup S. PPARs: fatty acid sensors controlling metabolism. Semin Cell Dev Biol 2012; 23(6): 631–639.

132. Prusty D, Park BH, Davis KE, Farmer SR. Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor (PPAR gamma) and C/EBP gene expression during the differentiation of 3T3-L1 preadipocytes. J Biol Chem 2002; 277(48): 46226–46232.

133. Rahman TM, Hodgson HJ. Animal models of acute hepatic failure. Int J Exp Pathol 2000; 81(2):145-157.

134. Rangwala SM, Rhoades B, Shapiro JS, Rich AS, Kim JK, Shulman GI, Kaestner KH, Lazar MA.

Geneticmodulation of PPAR phosphorylation regulates insulin sensitivity. Developmental Cell 2003; 5(4): 657-663.

135. Redondo S, Ruiz E, Santos-Gallego CG, Padilla E, Tejerina T. Pioglitazone induces vascular smooth muscle cell apoptosis through a peroxisome proliferator-activated receptor-gamma, transforming growth factor-beta1, and a Smad2-dependent mechanism. Diabetes 2005; 54(3): 811-817.

136. Reka AK, Kurapati H, Narala VR, Bommer G, Chen J, Standiford TJ, Keshamouni VG. Peroxisome proliferator-activated receptor-gamma activation inhibits tumormetastasis by antagonizing Smad3-mediated epithelial-mesencymal transition. Mol Cancer Ther 2010; 9(12): 3221-3232.

137. Ren X, Hogaboam C, Carpenter A, Colletti L. Stem cell factor restores hepatocyte proliferation in IL-6 knockout mice following 70% hepatectomy. J Clin Invest 2003; 112(9): 1407--1418.

138. Ribon V, Johnson JH, Camp HS, Saltiel AR. Thiazolidinediones and insulin resistance: peroxisome proliferator-activated receptor activation stimulates expression of the CAP gene. Proc Natl Acad Sci USA 1998; 95(25): 14751-14756.

139. Ribon V, Printen JA, Hofman NG, Kay BK, Saltiel AR. A novel, multifunctional c-Cbl binding protein in insulin receptor signaling in 3T3-L1 adipocytes. Molecular and Cellular Biology 1998; 15(1): 872–879.

140. Ricote M, Huang JT, Welch JS, Glass CK. The peroxisome proliferator-activated receptor (PPARgamma) as a regulator of monocyte/macrophage function. J Leukoc Biol 1999;66(5): 733-739.

141. Riehle KJ, Campbell JS, McMahan RS, Johnson MM, Beyer RP, Bammler TK, Fausto N. Regulation of liver regeneration and hepatocarcinogenesis by suppressor of cytokine signaling 3. J Exp Med 2008; 205(1): 91-103.

142. Rosen ED, Kulkarni RN, Sarraf P, Ozcan U, Okada T, Hsu CH, Eisenman D, Magnuson MA, Gonzalez FJ, Kahn CR, Spiegelman BM.. Targeted elimination of peroxisome proliferator-activated receptor γ in beta cells leads to abnormalities in islet mass without compromising glucose homeostasis. Mol Cell Biol 2003; 23(20): 7222-7229.

143. Ruan H, Pownall HJ, Lodish HF. Troglitazone antagonizes tumor necrosis factor-alpha-induced reprogramming of adipocyte gene expression by inhibiting the transcriptional regulatory functions of NF-kappaB. J Biol Chem 2003;278(30): 28181-28192.

144. Rudnick DA, Davidson NO. Functional relationships between lipid metabolism and liver regeneration. Int J

Hepatol 2012; 2012: 549241.

145. Sakamoto T, Liu Z, Murase N, Ezure T, Yokomuro S, Poli V, Demetris AJ. Mitosis and apoptosis in the liver of interleukin-6-deficient mice after partial hepatectomy. Hepatology 1999; 29(2): 403-411.

146. Sakata H, Takayama H, Sharp R, Rubin JS, Merlino G, LaRochelle WJ. Hepatocyte growth factor/scatter factor overexpression induces growth, abnormal development, and tumor formation in transgenic mouse livers. Cell Growth Differ 1996; 7(11): 1513-1523.

147. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism," Nature 2001; 414(6865): 799-806.

148. Schaefer KL, Wada K, Takahashi H, Matsuhashi N, Ohnishi S, Wolfe MM, Turner JR, Nakajima A, Borkan SC, Saubermann LJ. Peroxisome proliferator-activated receptor gamma inhibition prevents adhesion to the extracellular matrix and induces anoikis in hepatocellular carcinoma cells. Cancer Res 2005; 65 (6): 2251-2259.

149. Shimizu M, Hara A, Okuno M, Matsuno H, Okada K, Ueshima S, Matsuo O, Niwa M, Akita K, Yamada Y, Yoshimi N, Uematsu T, Kojima S, Friedman SL,Moriwaki H, Mori H. 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.

150. Shiota G, Kawasaki H. Hepatocyte growth factor in transgenic mice. Int J Exp Pathol 1998; 79(5): 267-277.

151. Shteyer E, Liao Y, Muglia LJ, Hruz PW, Rudnick DA. Disruption of hepatic adipogenesis is associated with impaired liver regeneration in mice. Hepatology 2004; 40(6): 1322-1332.

152. Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest 2000; 106(2): 171-176.

153. Smith U, Gogg S, Johansson A, Olausson T, Rotter V, Svalstedt B. Thiazolidinediones (PPAR agonists) but not PPAR agonists increase IRS-2 gene expression in 3T3-L1 and human adipocytes. FASEB Journal 2001; 15(1): 215–220.

154. Spector MS, Auer KL, Jarvis WD, Ishac EJ, Gao B, Kunos G, Dent P. Differential regulation of the mitogen-activated protein and stress-activated protein kinase cascades by adrenergic agonists in quiescent and regenerating adult rat hepatocytes. Mol Cell Biol 1997; 17(7): 3556-3565.

155. Srinivasan SR, Chow CK, Glauert HP. Effect of the peroxisome proliferator ciprofibrate on hepatic DNA

synthesis and hepatic composition following partial hepatectomy in rats. Toxicology 1990; 62(3): 321-332.

156. Standaert ML, Kanoh Y, Sajan MP, Bandyopadhyay P, Farese RV. Cbl, IRS-1, and IRS-2 mediate efects of rosiglitazone on PI3K, PKC-lambda and glucose transport in 3T3/L1 adipocytes. Endocrinology 2002; 143(5); 1705-1716.

157. Stolz DB, Mars WM, Petersen BE, Kim TH, Michalopoulos GK. Growth factor signal transduction immediately after two-thirds partial hepatectomy in the rat. Cancer Res 1999; 59(16): 3954-3960.

158. Sturm JW, Zhang H, Magdeburg R, Hasenberg T, Bönninghoff R, Oulmi J, Keese M, McCuskey R. Altered apoptotic response and different liver structure during liver regeneration in FGF-2-deficient mice. Cell Physiol Biochem 2004; 14(4-6): 249-260.

159. Suzawa M, Takada I, Yanagisawa J, Ohtake F, Ogawa S, Yamauchi T, Kadowaki T, Takeuchi Y, Shibuya H, Gotoh Y, Matsumoto K, Kato S. Cytokines suppress adipogenesis and PPAR-gamma function through the TAK1/TAB1/NIK cascade. Nat Cell Biol 2003; 5(3): 224-230.

160. Szanto A, Balint BL, Nagy ZS, Barta E, Dezso B, Pap A, Szeles L, Poliska S, Oros M, Evans RM, Barak Y, Schwabe J, Nagy L. STAT6 transcription factor is a facilitator of the nuclear receptor PPARγ-regulated gene expression in macrophages and dendritic cells. Immunity 2010; 33(5): 699–712.

161. Szanto A, Nagy L. The many faces of PPARgamma: anti-inflammatory by any means? Immunobiology 2008;213(9-10): 789-803.

162. Széles L, Töröcsik D, Nagy L. PPARγ in immunity and inflammation: cell types and diseases. Biochim Biophys Acta. 2007; 1771(8): 1014–1030.

163. Takada I, Kouzmenko AP, Kato S. PPAR-γ signaling crosstalk in mesenchymal stem cells. PPAR Res. 2010; 2010. Pii: :341671.

164. Talarmin H, Rescan C, Cariou S, Glaise D, Zanninelli G, Bilodeau M, Loyer P, Guguen-Guillouzo C, Baffet G. The mitogen-activated protein kinase kinase/extracellular signal-regulated kinase cascade activation is a key signalling pathway involved in the regulation of G1 phase progression in proliferating hepatocytes. Mol Cell Biol 1999; 19(9): 6003-6011.

165. Tang W, Zeve D, Seo J, Jo AY, Graff JM. Thiazolidinediones regulate adipose lineage dynamics. Cell Metab

2011; 14(1):116-122.

166. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol 2004; 5(10): 836-847.

167. Tietz PS, Larusso NF. Cholangiocyte biology. CCurr Opin Gastroenterol 2006; 22(3): 279-287.

168. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 1994; 79(7): 1147-1156.

169. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPAR promotes monocyte/macrophage diferentiation and uptake of oxidized LDL. Cell 1998; 93(2): 241-252.

170. Turmelle YP, Shikapwashya O, Tu S, Hruz PW, Yan Q, Rudnick DA. Rosiglitazone inhibits mouse liver regeneration. FASEB J 2006; 20(14): 2609-2611.

171. Ueki T, Kaneda Y, Tsutsui H, Nakanishi K, Sawa Y, Morishita R, Matsumoto K, Nakamura T, Takahashi H, Okamoto E, Fujimoto J. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. Nature Med 1999; 5(2): 226-230..

172. Viswakarma N, Jia Y, Bai L, Vluggens A, Borensztajn J, Xu J, Reddy JK. Coactivators in PPAR-Regulated Gene Expression. PPAR Res. 2010; 2010. pii: 250126.

173. Wahli W, Michalik L. PPARs at the crossroads of lipid signaling and inflammation. Trends Endocrinol Metab. 2012; 23(7): 351-363

174. Wakino S, Kintscher U, Kim S, Yin F, Hsueh WA, Law RE. Peroxisome proliferator-activated receptor gamma ligands inhibit retinoblastoma phosphorylation and G1--> S transition in vascular smooth muscle cells. J Biol Chem 2000; 275(29): 22435-22441.

175. Walldorf J, Hillebrand C, Aurich H, Stock P, Hempel M, Ebensing S, Fleig WE, Seufferlein T, Dollinger MM, Christ B. Propranolol impairs liver regeneration after partial hepatectomy in C57Bl/6-mice by transient attenuation of hepatic lipid accumulation and increased apoptosis. Scand J Gastroenterol 2010; 45(4):468-476.

176. Wang X, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Grompe M. The origin and liver repopulating capacity of murine oval cells. Proc Natl Acad Sci USA 2003; 100 Suppl 1: 11881-11888.

177. Webber EM, Wu JC, Wang L, Merlino G, Fausto N. Overexpression of transforming growth factor-alpha causes liver enlargement and increased hepatocyte proliferation in transgenic mice. Am J Pathol 1994; 145(2):

398–408.

178. Weir E, Chen Q, DeFrances MC, Bell A, Taub R. Zarnegar R. Rapid induction of mRNAs for liver regeneration factor and insulin-like growth factor binding protein-1 in primary cultures of rat hepatocytes by hepatocyte growth factor and epidermal growth factor. Hepatology 1994; 20(4 Pt 1): 955-960.

179. Weymann A, Hartman E, Gazit V, Wang C, Glauber M, Turmelle Y, Rudnick DA. p21 Is required for dextrose-mediated inhibition of mouse liver regeneration. Hepatology 2009; 50(1): 207-215.

180. Wheeler MD, Smutney OM, Check JF, Rusyn I, Schulte-Hermann R, Thurman RG. Impaired Ras membrane association and activation in PPARalpha knockout mice after partial hepatectomy. Am J Physiol Gastrointest Liver Physiol 2003; 284(2):G302-G312.

181. Willson TM, Cobb JE, Cowan DJ, Wiethe RW, Correa ID, Prakash SR, Beck KD, Moore LB, Kliewer SA, Lehmann JM. The structure-activity relationship between peroxisome proliferator- activated receptor gamma agonism and the antihyperglycemic activity of thiazolidinediones. J Med Chem 1996; 39(3): 665-668.

182. Wuestefeld T, Pesic M, Rudalska R, Dauch D, Longerich T, Kang TW, Yevsa T, Heinzmann F, Hoenicke L, Hohmeyer A, Potapova A, Rittelmeier I, Jarek M,Geffers R, Scharfe M, Klawonn F, Schirmacher P, Malek NP, Ott M, Nordheim A, Vogel A, Manns MP, Zender L. A direct in vivo RNAi screen identifies MKK4 as a key regulator of liver regeneration. Cell 2013; 153(2): 389-401.

183. Wustefeld T, Rakemann T, Kubicka S, Manns MP, Trautwein C. Hyperstimulation with interleukin 6 inhibits cell cycle progression after hepatectomy in mice. Hepatology 2000; 32(3) :514-522.

184. 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 USA 1997; 94(4): 1441-1446.

185. Yamamoto Y, Ono T, Dhar DK, Yamanoi A, Tachibana M, Tanaka T, Nagasue N. Role of peroxisome proliferator-activated receptor-gamma (PPARgamma) during liver regeneration in rats. J Gastroenterol Hepatol 2008; 23(6): 930-937.

186. Yang FG, Zhang ZW, Xin DQ, Shi CJ, Wu JP, Guo YL, Guan YF. Peroxisome proliferator-activated receptor gamma ligands induce cell cycle arrest and apoptosis in human renal carcinoma cell lines. Acta Pharmacol Sin 2005; 26(6): 753-761.

187. Yeoh GC, Ernst M, Rose-John S, Akhurst B, Payne C, Long S. Opposing roles of gp130-mediated STAT-3 and ERK-1/2 signaling in liver progenitor cell migration and proliferation. Hepatology 2007; 45(2): 486--494.

188. Yu J, Shen B, Chu ES, Teoh N, Cheung KF, Wu CW, Wang S, Lam CN, Feng H, Zhao J, Cheng AS, To KF, Chan HL, Sung JJ. Inhibitory role of peroxisome proliferator-activated receptor gamma in hepatocarcinogenesis in mice and in vitro. Hepatology 2010; 51(6): 2008-2019.

189. Yuan X, Yan S, Zhao J, Shi D, Yuan B, Dai W, Jiao B, Zhang W, Miao M. Lipid metabolism and peroxisome proliferator-activated receptor signaling pathways participate in late-phase liverregeneration. J Proteome Res 2011; 10(3):1179-1190.

190. Zhang L, Wang YD, Chen WD, Wang X, Lou G, Liu N, Lin M, Forman BM, Huang W. Promotion of liver regeneration/repair by farnesoid X receptor in both liver and intestine in mice. Hepatology 2012; 56(6):2336-2343.

191. Zingarelli B, Sheehan M, Hake PW, O'Connor M, Denenberg A, Cook JA. Peroxisome proliferator activator receptor-gamma ligands, 15-deoxy-Delta(12,14)-prostaglan-din J2 and ciglitazone, reduce systemic inflammation in polymicrobial sepsis by modulation of signal transduction pathways. J Immunol 2003; 171(12): 6827–6837.

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