

ATVB IN FOCUS: Plasminogen Activation in Disease



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Role of the Plasminogen Activation System in Liver Injury and Repair: Knowns and Known Unknowns

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ABSTRACT: The plasminogen activation system (PAS) is primarily responsible for the degradation of fibrin clots as well as extracellular matrix remodeling. Many components of the PAS are produced by the liver, and the expression of these proteins is altered during liver disease. Liver is a primary site for the synthesis of proteins in the PAS, including plasminogen, tPA (tissue-type plasminogen activator), and uPA (urokinase-type plasminogen activator), as well as several modulators of plasmin activation. Clinical studies have shown that liver injury profoundly changes levels of PAS components and fibrinolytic activity in plasma. Likewise, there is strong experimental evidence to suggest that components of the PAS play an important role in determining the severity of hepatic injury and, paradoxically, also contribute to repair and regeneration of the injured liver. Here, we review the dynamic connections between the PAS and liver injury/disease, including changes in PAS expression and function accompanying liver disease in patients and mechanism-centered studies in animal models. We focus on established models of acute hepatic injury, chronic liver disease, and repair/regeneration, and review the effect of gain or loss of function of PAS components on the liver. The review seeks to cover not only field-driving discoveries but also spotlights unexplained dichotomies, challenges with interpretation, and the need for further exploration of mechanisms using leading-edge tools. Critical gaps in our understanding of how the PAS contributes to liver pathophysiology, and vice versa, are identified, and future directions are considered.

GRAPHIC ABSTRACT: A [graphic abstract](#) is available for this article.

Key Words: fibrinolysis ■ liver ■ liver diseases ■ plasminogen ■ plasminogen activators

The coagulation cascade is activated in response to tissue injury. Activation of coagulation leads to an amplified series of proteolytic reactions, ultimately generating the serine protease thrombin. Thrombin cleaves circulating fibrinogen to form fibrin, which deposits at sites of vascular injury and tissue damage. Fibrin formation is counterbalanced by fibrin degradation, a process termed fibrinolysis. The primary fibrinolytic protease, plasmin, is generated from its zymogen, plasminogen, which circulates in plasma. The plasminogen activators, tPA (tissue-type plasminogen activator) and uPA (urokinase-type plasminogen activator), proteolytically convert

plasminogen to its active form.¹ Plasmin generation and activity are regulated by multiple mechanisms. tPA and uPA activity are inhibited by the PAI (plasminogen activator inhibitor)-1 and PAI-2. uPAR (urokinase-type plasminogen activator receptor) also regulates the plasminogen activation system (PAS) by binding uPA at the cell surface.² In addition, TAFI (thrombin-activatable fibrinolysis inhibitor, carboxypeptidase B2) inhibits plasmin-mediated

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Nonstandard Abbreviations and Acronyms

α2-AP	α2-antiplasmin
ALI/ALF	acute liver injury or failure
APAP	acetaminophen/paracetamol
CCl₄	carbon tetrachloride
HCC	hepatocellular carcinoma
HGF	hepatocyte growth factor
MASH	metabolic dysfunction–associated steatohepatitis
MASLD	metabolic dysfunction–associated steatotic liver disease
PAI	plasminogen activator inhibitor
PAP	plasmin-α2-antiplasmin
PAS	plasminogen activation
PHx	partial hepatectomy
Plg	plasminogen gene
TAFI	thrombin-activatable fibrinolysis inhibitor
tPA	tissue-type plasminogen activator
uPA	urokinase-type plasminogen activator
uPAR	urokinase-type plasminogen activator receptor

fibrinolysis by cleaving plasmin binding sites on fibrin.^{1,3} Plasmin is also inhibited by α2-AP (α2-antiplasmin), which directly binds to plasmin, forming an irreversible PAP (plasmin-α2-antiplasmin) complex, preventing plasmin from interacting with fibrin. α2-AP is crosslinked to fibrin by coagulation factor XIII, disrupting local fibrinolysis and stabilizing fibrin. Importantly, precise regulation of plasmin activity ensures proper timing of fibrin clot clearance and avoids improper accelerated removal of fibrin clots.

Because the liver is the site of synthesis of multiple key components of the hemostatic system, patients with hepatic dysfunction develop significant alterations in coagulation and fibrinolysis, as well as regulation of platelet functions.^{4–7} Strong evidence suggests that the PAS and fibrinolysis are also altered in patients with acute liver injury and chronic liver disease.^{4,8} These alterations may further complicate the clinical management of hemostatic and thrombotic dysregulation in patients with liver disease. Likewise, components of the PAS have been linked to the pathogenesis of liver disease itself, including mechanisms independent of traditional plasmin-directed fibrinolytic activity, such as activation of proinflammatory pathways.³ These interactions make for a complex reciprocal interface between the PAS and liver damage and dysfunction. Here, we review clinical and experimental evidence bidirectionally linking hepatic injury and dysfunction to changes in systemic fibrinolysis and elucidating mechanisms whereby primary PAS components contribute to liver damage, repair, and regeneration. Where relevant, we highlight connections to clinical observations, gaps in the literature, and note where context-dependent

Highlights

- Current experimental and clinical studies highlight the importance of the plasminogen activation system in acute and chronic liver disease, as patients with hepatic dysfunction develop significant changes in coagulation and fibrinolysis.
- Cause of liver damage and phase of injury/repair seem to influence the impact of the plasminogen activation system on the liver.
- The complex, bidirectional relationship between liver dysfunction and fibrinolysis highlights the need for more mechanistic studies and new approaches, including emerging in vivo tools.

contributions of components of the PAS to liver injury leave unresolved dichotomies evident in the literature.

RECIPROCAL CONNECTIONS BETWEEN ACUTE LIVER INJURY AND THE PAS

Impact of Acute Hepatic Injury on Fibrinolysis

The liver consists of both parenchymal cells (ie, hepatocytes) and several different nonparenchymal cells, including specialized sinusoidal endothelial cells, resident liver macrophages (ie, Kupffer cells), and hepatic stellate cells. Acute liver injury or failure (ALI/ALF), including hepatocyte damage and dysfunction, is driven by multiple causes, including autoimmune hepatitis and drug toxicity.⁹ ALI/ALF is characterized by a sudden and rapid decline of hepatic function, which may be secondary to a chronic liver condition or caused by pathogenic infection, toxin exposure, or an adverse drug reaction. Prior studies have examined the impact of ALI/ALF on systemic levels and activation of PAS components. Compared with healthy controls, plasma levels of plasminogen, TAFI, and α2-AP are significantly decreased in patients with ALI/ALF.⁴ This change may be a consequence of decreased expression of these proteins by the injured liver or consumption of existing factors. Indeed, plasma levels of PAP complexes, a biomarker of plasmin production, increase in patients with ALI/ALF.¹⁰ Notably, plasma tPA and PAI-1 levels increase in the plasma of patients with ALI/ALF.⁴ When functional assays are used to understand the net effect of these changes on fibrinolysis, plasma clots from patients with hepatic injury display marked changes. For example, prolonged ex vivo clot lysis time was observed in patients with ALI/ALF.^{4,11} These studies highlight how combined disruption of the synthesis of PAS components alongside injury-induced plasmin generation can contribute to substantial alterations in systemic fibrinolysis. Although higher plasma clot lysis time was associated with increased disease severity, notably, there was no association between ex vivo clot lysis time and transplant-free survival in patients

with ALF.¹¹ Whereas the capacity to generate plasmin and lyse clots suggested by ex vivo experiments is not connected to an outcome of transplant-free survival in patients, it is unclear whether plasmin generated in the liver in vivo is connected to the outcome by contributing to injury progression or resolution.

Acetaminophen Overdose–Induced Acute Liver Injury

A leading cause of ALI/ALF is acetaminophen/paracetamol (APAP) overdose.^{12,13} Interestingly, 1 study suggests a decrease in circulating plasminogen concentration in individuals susceptible to APAP-induced liver injury, even at therapeutic doses of APAP.¹⁴ In addition to several studies linking hepatic injury to altered fibrinolysis in patients, experimental studies suggest potential mechanistic links between fibrinolysis and acute liver damage. APAP overdose in mice provides a robust and reproducible experimental setting of acute liver injury for which the associated mechanisms and liver pathology resemble those observed in cultured primary human hepatocytes and in patients.^{15,16} Although the kinetics of APAP metabolism and injury development vary, and sex differences with known mechanistic origins are known,¹⁷ this versatile model, paired with studies in human hepatocytes, has formed the basis for our understanding of APAP hepatotoxicity. APAP is metabolized to a reactive metabolite, *N*-acetyl-*p*-benzoquinone imine, which, if generated in excess, triggers intracellular oxidative stress, mitochondrial dysfunction, and cell signaling pathways that prompt hepatocyte injury.¹⁸ Damage to hepatocytes induced by APAP occurs in concert with multiple extrahepatocellular pathways that contribute to the progression of hepatocellular necrosis in this model.¹⁹ Hepatic plasmin activity is reported to increase in mice after APAP overdose, corresponding temporally with expression of tPA.²⁰ Likewise, in vivo plasmin generation is indicated by PAP complex formation in APAP-challenged mice, an observation reflected by increased plasma PAP complexes in patients.¹⁰ Several studies suggest plasmin contributes to some aspect of APAP-induced liver injury in mice, but there are important differences in these studies that are worth noting. Two separate studies suggest APAP-induced hepatocyte damage, marked by changes in hepatocellular necrosis and liver enzymes, is reduced in genetic plasminogen knockout (*Plg*^{−/−}) mice.^{10,20,21} Another similar experiment identified a reduction in sinusoidal endothelial cell injury/vascular dysfunction in *Plg*^{−/−} mice, but hepatocyte injury was not substantially impacted.²⁰ The precise basis for these discrepancies is not known, but *Plg*^{−/−} mice display baseline phenotypes including hepatic fibrin deposition, which could vary in severity in individual colonies, potentially impacting outcomes in liver injury models. Aligning with that result, peak hepatotoxicity is minimally reduced by tranexamic acid in APAP-challenged mice.²¹

Prior studies suggest that excess plasmin activity during the development of hepatotoxicity drives vascular injury and hepatic congestion, an impairment of blood drainage from the liver, after APAP overdose.²⁰ Within 6 hours of APAP challenge, hepatic PAI-1 mRNA and plasma PAI-1 levels increased.²² Importantly, the cellular source of PAI-1 in APAP-challenged mice has not been identified, although recent single-cell RNA sequencing documents robust *Serpine 1* (PAI-1) expression by hepatic stellate cells.²³ PAI-1 deficiency dramatically increased hepatic congestion and hepatocyte injury in APAP-challenged mice.^{21,24} Exaggerated hepatic congestion in APAP-challenged *PAI-1*^{−/−} mice was largely mitigated by the concurrent absence of tPA.²¹ Separately, administration of exogenous recombinant PAI-1-insensitive tPA (ie, tenecteplase) to APAP-challenged wild-type mice produced a histological picture resembling massive intrahepatic congestion reminiscent of APAP-challenged *PAI-1*^{−/−} mice.²¹ However, a single deficiency in tPA did not impact APAP-induced liver injury.²⁵

The impact of deficiency in components of the PAS may be perceived to have opposing roles, depending on the time point examined. For example, TAFI deficiency reduces early APAP hepatotoxicity, but as injury progresses, hepatic necrosis is exaggerated in APAP-challenged *TAFI*^{−/−} mice.²⁶ Moreover, missing from mechanistic studies is a complete understanding of the plasmin targets that drive hepatic congestion (and liver damage). Whereas Occam's razor would suggest that degradation of fibrin would be a frontline mechanism, multiple studies suggest that fibrin(ogen) plays a hepatoprotective role in APAP-induced liver injury (by providing structure and engaging leukocytes²⁷) at least at doses associated with acute injury and repair.²⁸ Indeed, at larger doses that produce ALF-like features, biomarkers of fibrinolysis are markedly exaggerated.²⁹ Moreover, there is at least 1 example where a fibrin(ogen) mutation drives hepatic injury by enhancing fibrinolysis.³⁰

Other Experimental Models of Acute Liver Injury

Contributions of the PAS to liver injury have also been examined in other experimental settings. Collectively, these studies demonstrate that experimental context matters and that the basis of the hepatic insult can dictate involvement of the PAS. Administration of the solvent carbon tetrachloride (CCl₄) is among the most widely used models of chemical-induced acute liver damage and fibrosis, either alone as a hepatotoxic challenge or paired with overnutrition to magnify hepatic fibrosis in the context of steatohepatitis.^{31,32} Like APAP, CCl₄ challenge produces centrilobular hepatic necrosis, but through mechanisms involving free radical formation and marked lipid peroxidation.³³ PAI-1 deficiency exacerbates hepatic injury in CCl₄-challenged mice,³⁴ implying that plasmin activity may worsen hepatic injury. Despite

this observation, tPA deficiency does not impact CCl₄-induced hepatotoxicity.³⁵ Likewise, *Plg*^{-/-} mice are similarly sensitive to CCl₄-induced liver damage,³⁶ a notable dichotomy from APAP-induced liver injury, where several studies suggest hepatic congestion or hepatic injury is reduced in *Plg*^{-/-} mice.

Robust PAI-1 induction is observed in rodents challenged with lipopolysaccharide, and in models where low-dose lipopolysaccharide is administered to potentiate the hepatotoxicity of another chemical.³⁷ In this case, PAI-1 deficiency may reduce hepatic injury by mechanisms beyond fibrinolysis, as *PAI-1*^{-/-} mice had reduced plasma concentrations of several cytokines, suggesting a proinflammatory, nonfibrinolytic role of PAI-1.³⁸ PAI-1 has also been examined in settings of cholestatic liver injury, a subtype of acute liver injury in which bile flow is disrupted. Several studies have investigated the role of plasminogen activation and inhibition in cholestatic liver injury, with a particular focus on PAI-1 knockout mice.³⁹⁻⁴² Interestingly, PAI-1 deficiency dramatically reduced hepatic injury in mice after experimental bile duct ligation.³⁹ *PAI-1*^{-/-} mice with bile duct ligation displayed reduced hepatic injury and inflammation and a commensurate reduction in extracellular matrix deposition.^{39,42} This suggests that PAI-1 deficiency reduces hepatic injury in the context of obstructive cholestasis. Conversely, tPA-deficient mice displayed increased hepatocyte death, increased neutrophil infiltration, and larger bile infarcts.⁴⁰ Notably, although there is a clear impact of tPA or PAI-1 deficiency in mice after bile duct ligation, the precise role of fibrinogen and fibrinolysis has not been examined, leaving the precise mechanisms not fully understood.

FIBRINOLYSIS IN LIVER REPAIR AND REGENERATION

Plasmin generation plays a key role in the clearance of provisional fibrin matrix deposited at sites of tissue damage and is thus closely linked to wound healing. Although tight control of plasmin production is critical during early liver injury, there is also strong evidence that components of the PAS play a key role in liver repair and resolution of hepatic necrosis. For example, whereas deficiency in uPA or plasminogen does not impact acute CCl₄ hepatotoxicity,⁴³ there is a dramatic delay in the clearance of necrotic material from the injured liver in the absence of plasminogen,⁴⁴ with centrilobular lesions still present 30 days after injury. Similar observations have been made in APAP-challenged mice. For example, delayed administration of tranexamic acid (ie, near peak hepatotoxicity) delayed repair of the APAP-injured liver, despite limited to no effect of tranexamic acid on peak APAP hepatotoxicity.⁴⁵ This study highlights how the role of plasmin may evolve over time after a hepatotoxic challenge.

Notably, the precise mechanism whereby plasmin(ogen) drives liver repair is not known. Fibrin(ogen) accumulation in the injured liver is exaggerated in the absence of plasminogen, and a failure to clear the fibrin matrix could impair injury resolution. However, superimposing fibrinogen deficiency did not impact injury resolution in CCl₄-challenged plasminogen-deficient mice.³⁶ This observation led others to propose the hypothesis that clearance of nonfibrin matrix or an alternative mechanism is central to plasmin-directed liver repair.^{36,46} Indeed, the activation of signaling pathways promoting prorepair macrophage activation may also participate.^{45,47}

In addition to a critical role in resolving hepatocellular necrosis caused by toxicants and hepatotoxic drugs, components of the fibrinolytic system are also linked to the regeneration of the liver. Coordinated proliferation of liver cells via complex cellular crosstalk and signaling pathways⁴⁸ is required to restore liver mass after liver surgery and in the context of liver transplantation. Partial liver resection (ie, partial hepatectomy, PHx) is a routine surgical procedure used to treat various liver diseases, including resection of liver tumors.⁴⁹ Strong clinical evidence indicates alterations in fibrinolysis after PHx, and there is experimental evidence linking components of the fibrinolytic system to liver regeneration. Mechanisms driving liver regeneration have been uncovered using standard 70% (two-thirds) PHx in both rats and mice.⁵⁰ Removal of liver mass using this experimental approach is followed swiftly by liver parenchymal proliferation, restoration of nonparenchymal cell architecture and angiogenesis, and tissue matrix remodeling, ultimately restoring liver mass on a predictable timeline (ie, ≈2 weeks post-PHx).⁵¹

Notably, activation of the fibrinolytic system is often highlighted as one of the earliest molecular events in the remnant liver in both experimental two-thirds PHx and in patients.⁵²⁻⁵⁵ Increased hepatic uPA activity occurred within minutes after two-thirds PHx in rats, and multiple studies document reduced hepatocyte proliferation and delayed restoration of liver mass in *uPA*^{-/-} mice.^{56,57} One of the proposed mechanisms whereby uPA drives liver regeneration is through activation of HGF (hepatocyte growth factor), an essential mitogen for hepatocyte proliferation.⁵⁸ Prior studies suggest this regulation is reciprocal, as HGF also increases uPA production.^{59,60} Notably, the proposed mechanism whereby uPA activates HGF to promote liver regeneration is a combined impression of in vitro observations and the observed impact of uPA deficiency on PHx-induced liver regeneration in mice. The precise mechanisms whereby uPA contributes to liver regeneration are not known, and there is no direct evidence that uPA promotes liver regeneration through activation of HGF. Because uPA activates plasminogen, another longstanding hypothesis emphasizes the role of plasminogen in facilitating liver regeneration. Plasminogen deficiency has been associated with impaired hepatocyte proliferation and liver regeneration after PHx.^{61,62} Key

questions remain as to the precise role of the fibrinolytic system in liver regeneration. Moreover, there is a need to resolve several paradoxical observations that seem counter-current to a simple mechanism whereby uPA directly or indirectly drives liver regeneration. For example, PAI-1 deficiency, which would be anticipated to increase uPA activity, attenuates hepatocyte proliferation after PHx in mice, as does treatment with small molecule antagonists of fibrin-integrin engagement.⁶³ Likewise, the hypothesis that plasminogen is a key driver of liver damage is at odds with the observation that hepatic fibrin(ogen) deposits seem to drive liver regeneration.⁶⁴ Indeed, intraoperative hepatic fibrin(ogen) deposition is reported to fail in patients who develop posthepatectomy liver failure, a major complication of failed liver regeneration in patients undergoing partial liver resection.⁵⁰ Overall, multiple paradoxical aspects of known connections between fibrinolytic enzymes (and fibrinogen) and liver regeneration highlight the need to uncover the precise mechanisms linking uPA and plasminogen to liver regeneration.

FIBRINOLYSIS IN CHRONIC LIVER DISEASE

The primary drivers of chronic liver disease are wide-ranging and include hepatitis, autoimmunity, genetic disorders, alcohol consumption, and metabolic dysfunction-associated steatohepatitis (MASH). The latter 2 are major causes of liver disease, given the advent of new treatments for hepatitis C. Chronic liver disease of any cause poses a significant disease burden, with an estimated 1.5 billion people with these conditions globally.⁶⁵ Regardless of cause, unchecked liver disease not counterbalanced by liver repair can put patients at risk of developing hepatic fibrosis. Chronic liver damage drives phenotypic conversion of hepatic stellate cells to a myofibroblast-like phenotype, and these cells deposit excess matrix proteins (eg, collagen), leading to fibrosis of the liver.⁶⁶ In patients with severe chronic liver disease, functional liver mass is reduced because of scarring. A common misconception is that hepatic dysfunction in patients with severe liver disease uniformly predisposes patients to bleeding. This view has been replaced by evidence of a delicately rebalanced hemostatic state in patients with cirrhosis, reviewed elsewhere in detail.⁶ Because the liver plays a key role in the synthesis of multiple components of the plasminogen activator system, including plasminogen, the plasminogen activators, and other proteins, including TAFI, α 2-AP, and PAI-1, cirrhotic patients often display altered plasma levels of these components and either hypofibrinolysis or hyperfibrinolysis.⁸ Beyond alterations in systemic fibrinolysis, these changes may also contribute to the pathogenesis of liver disease by influencing inflammatory processes and cellular effector functions. This section briefly covers the interplay

between the plasminogen activating system and chronic liver disease, focusing primarily on evidence linking changes in fibrinolytic enzymes to disease pathogenesis.

Several studies suggest that components of the PAS contribute to the development of chronic liver disease. Metabolic dysfunction-associated steatotic liver disease (MASLD) is among the most common causes of chronic liver disease and is a hepatic manifestation of obesity. Progression of hepatic steatosis to steatohepatitis can ultimately lead to hepatic fibrosis, cirrhosis, and liver cancer. Prior studies suggest connections between components of the PAS and mechanisms driving the progression of MASLD. Expression of PAI-1 is associated with obesity and prior studies identify PAI-1 as an adipokine, whereupon a reduction in adiposity reduces PAI-1 levels.⁶⁷ PAI-1 deficiency or inhibition reduces hepatic steatosis in obese mice,^{68–70} although these effects may be transient and may not completely prevent progression to MASH and fibrosis.⁷¹ Notably, assumptions that adipocytes are the primary pathological source of PAI-1 should perhaps be revisited, as recent studies identify hepatic stellate cells as a relevant source of PAI-1 expression in experimental MASLD.⁷² Liver-targeted therapeutic approaches to reduce PAI-1 expression may ultimately form the foundation for novel strategies to treat MASLD (or obesity). The precise mechanisms linking PAI-1 to obesity-associated dysmetabolism, including MASLD/MASH, are not known. These mechanisms may involve direct connections to plasminogen—in a study of diet-induced steatohepatitis, plasminogen deficiency and pharmacological plasmin inhibition by tranexamic acid appeared to reduce hepatic fibrosis associated with MASH,⁷³ although this has not been explored clinically. In addition, emerging evidence suggests important interactions between tPA, PAI-1, and lipoprotein metabolism—regulation of tPA and PAI-1 and lipoproteins are closely linked, and imbalance of both systems is implicated in dyslipidemia and atherosclerotic conditions.⁷⁴ Indeed, multiple components of the PAS may participate in the pathogenesis of chronic liver diseases like MASLD by mechanisms independent of canonical links to plasminogen, an important observation particularly when interpreting seemingly dichotomous results.

In addition to MASLD, there is strong evidence linking PAI-1 to the pathogenesis of alcoholic liver disease.⁷⁵ PAI-1-deficient mice displayed reduced liver pathology in experimental settings of alcohol-induced liver disease and in potentiation models in which the pathological effects of alcohol are exacerbated by coadministration of lipopolysaccharide.^{76,77} Like experimental MASLD, hepatic stellate cells may be a relevant source of PAI-1 in the context of alcoholic liver disease.⁷⁸ Unlike MASLD, however, there is a curious paucity of studies evaluating the impact of PAI-1 inhibitors on alcohol-induced liver pathology, despite strong protection in genetic models.

The PAS may contribute to the development of hepatic fibrosis, although prior studies do not offer a unified conclusion on whether the role of the PAS is profibrotic or antifibrotic. For example, PAI-1 deficiency exacerbates hepatic fibrosis in experimental CCl₄-induced liver fibrosis, but this likely represents a pathological extension of the exaggerated hepatotoxic effects of CCl₄ in PAI-1 knockout mice.³⁴ Although PAI-1 deficiency would be anticipated to enhance tPA activity, one study found that tPA deficiency increased hepatic fibrosis in CCl₄-challenged mice.⁷⁹ Validation of this observation led to the identification of potential mechanisms whereby tPA inhibits myofibroblast activation of hepatic stellate cells.³⁵ Indeed, as key players in the pathogenesis of chronic liver disease, stellate cells are long known to express components of the PAS.⁸⁰ Likewise, PAI-1 deficiency reduces hepatic fibrosis in mice after bile duct ligation, an observation connected to reduced hepatic injury. Other studies have shown that, like CCl₄-induced liver fibrosis, PAI-1 deficiency exacerbates bile duct fibrosis in mice exposed to another hepatotoxicant that selectively targets epithelial cells lining intrahepatic bile ducts.⁸¹ Beyond these models of chemical-induced tissue fibrosis, there is a need for additional studies evaluating the role of the PAS in fibrosis driven by common causes, including MASLD. Because the PAS may contribute to multiple aspects of disease pathology, it may be difficult to differentiate effects on fibrosis from upstream impact on dysmetabolism.

Advanced fibrosis and cirrhosis driven by chronic liver diseases, such as alcoholic liver disease and MASH, as well as hepatitis B and C infections, are all risk factors for the development of hepatocellular carcinoma (HCC).⁸² HCC is one of the most prevalent types of cancer worldwide, for which both the rate of incidence and mortality continue to rise.⁸³ Late diagnosis and treatment of HCC drastically reduce survival,⁸² highlighting the need to identify biomarkers for detection and develop targeted therapies. In HCC cells in culture, PAI-1 upregulation is indicative of poor prognosis and increased malignancy.⁸⁴ This is corroborated by a study in postcurative resection patients, in which high PAI-1 expression correlated significantly with poor survival, and PAI-1 expression was significantly higher in areas of HCC tissue than adjacent liver tissue.⁸⁵ This suggests that inhibition of plasminogen activation contributes to the malignancy of HCC. However, HCC cells in culture have been found to have increased uPA expression, and inhibition of uPA activation decreased HCC cell growth in vivo.⁸⁶ The complexities of plasminogen activation and inhibition in HCC must be further studied to underpin the fibrinolysis-related mechanisms by which cancerous cells in the liver proliferate.

SUMMARY AND PERCEIVED NEEDS

Because the liver is a key site of synthesis of numerous components of the PAS, acute and chronic liver damage are associated with systemic changes in PAS activity

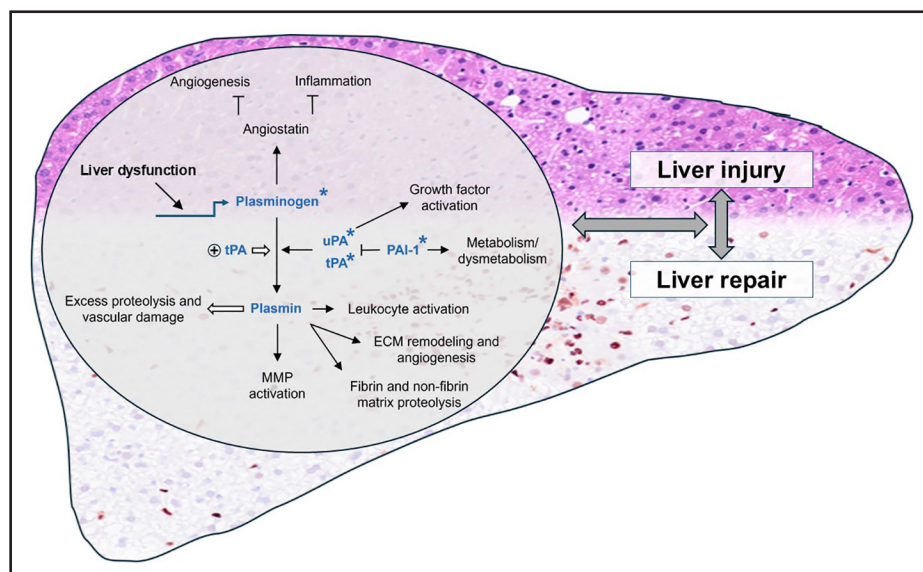


Figure. Reciprocal role and potential mechanisms linking the plasminogen activation system (PAS) to liver injury and liver repair.

Graphical summary of mechanisms linking plasmin(ogen) to liver injury and disease. Asterisks indicate the primary components of the PAS. Strong evidence links plasmin(ogen) and related elements of the PAS to liver injury and repair. Multiple studies suggest that excess plasmin(ogen) activation can exacerbate acute liver damage and contribute to pathologies common to chronic liver disease. At the same time, several lines of experimentation document a critical role for plasmin(ogen) and the PAS in liver repair and regeneration. Critical determinants of the precise role of PAS may include the cause of liver damage (ie, the experimental model or disease type), time point examined, and efficacy/specificity of the experimental interventions used to modify PAS function. ECM indicates extracellular matrix; MMP, matrix metalloproteinase; PAI, plasminogen activator inhibitor; tPA, tissue-type plasminogen activator; and uPA, urokinase-type plasminogen activator.

and fibrinolysis. Liver damage itself likely contributes to the consumption of PAS components, which, paired with impaired synthesis, magnifies these changes relative to other pathologies in which hepatic synthesis is unaffected. Moreover, the liver is increasingly recognized as an important source of key regulators of the PAS, such as PAI-1, more traditionally thought of as adipokines. As much as hepatic injury and disease can induce changes in systemic levels and activity of the PAS, components of PAS have been shown in multiple studies to alter the extent of acute liver damage and progression of chronic liver disease. Moreover, the PAS has been linked to liver repair, highlighting the complexity of the mechanisms linking liver disease with fibrinolysis.

A review of the literature highlights several points, as reinforced in the Figure and the Table. Although there are some similarities across models of liver damage and repair with respect to contributions of the PAS, the cause of liver damage and phase of injury/repair seem to influence the impact of the PAS on the liver. Although some mechanistic conclusions seem well-supported, generalizations are challenging to form amid the literature, and mechanistic details are lacking. In part, this may relate to the balance of strengths and limitations of the tools used to evaluate the mechanisms connecting the PAS to liver disease. Several use well-established mouse models with germline deficiencies in Plg, uPA, or tPA.

Although congenital plasminogen deficiency in mice does not affect reproduction or survival at baseline,⁸⁷ the *Plg*^{-/-} model highlights the multisystemic effects of plasminogen deficiency. Compared with age-matched control mice, *Plg*^{-/-} mice exhibit rectal lesions, hepatic lesions, and fibrin deposition in multiple tissues, including the liver.⁸⁷ uPA deficiency can also result in fibrin deposition, with combined tPA and uPA deficiency being more severe.⁸⁸ These phenotypic consequences, particularly those associated with baseline hepatic fibrin formation, should be included within the interpretation of results in models specifically focused on liver pathology. Complementary approaches, such as pharmacological inhibition of plasminogen, often do not produce the same impact as complete Plg deficiency. Although this certainly may relate to an incomplete inhibition of plasminogen, off-target effects of these drugs are also worth considering. Emerging tools that allow substantial inducible plasminogen depletion in adult animals may offer another complementary approach. For example, small interfering RNA-induced plasminogen depletion in mice did not induce fibrin deposition, weight loss, or elevation in hepatic expression of proinflammatory markers.⁸⁹

Particularly because of the complex interplay between liver dysfunction and fibrinolysis, it is important that the mechanisms linking the PAS to liver damage be uncovered. Unresolved dichotomy even in standard

Table. Impact of Experimental PAS Interventions on Acute Liver Injury, Liver Repair/Regeneration, and Chronic Liver Disease

Effects of PAS intervention in liver dysregulation							
Experimental setting		PAS intervention					
		TXA	<i>Plg</i> ^{-/-} mice	<i>uPA</i> ^{-/-} mice	<i>tPA</i> ^{-/-} mice	<i>PAI-1</i> ^{-/-} mice	<i>TAFI</i> ^{-/-} mice
Acute liver injury	APAP overdose	Minimal impact on hepatotoxicity ²¹	↓ Hepatocyte injury, ^{10,21} ↓ vascular injury ²⁰		No impact on hepatotoxicity ²⁵	↑ tPA-dependent hepatocyte injury and congestion ^{21,24}	↓ Early hepatotoxicity, ↑ hepatocyte injury with progression ²⁶
	BDL				↑ Hepatocyte injury ⁴⁰	↓ Hepatocyte injury ^{39,42}	
	CCl ₄		No impact on hepatotoxicity ⁴⁴	No impact on hepatotoxicity ⁴³	No impact on hepatotoxicity ³⁵	↑ Hepatocyte injury ³⁴	
Liver repair/regeneration	APAP overdose	Delayed repair ⁴⁵					
	CCl ₄		Delayed repair ⁴⁴	Delayed repair ⁴³			
	PHx		↓ Hepatocyte proliferation and restoration of liver mass ^{61,62}	↓ Hepatocyte proliferation and restoration of liver mass ^{56,57}		↓ Hepatocyte proliferation ⁶³	
Chronic liver damage	CCl ₄		No impact on hepatotoxicity ³⁶		↑ Hepatic fibrosis ⁷⁹	↑ Hepatic fibrosis ³⁴	
	MASLD/MASH	↓ Hepatic fibrosis ⁷³				↓ Hepatic steatosis ^{69,70}	

Summary of effects of PAS interventions, particularly genetic knockout mouse models, on experimental settings of acute liver injury, liver repair/regeneration, and chronic liver disease. Blank boxes in the table indicate intersections for which there are no published studies included in our review. APAP indicates acetaminophen/paracetamol; BDL, bile duct ligation; CCl₄, carbon tetrachloride; MASH, metabolic dysfunction–associated steatohepatitis; MASLD, metabolic dysfunction–associated steatotic liver disease; PAI, plasminogen activator inhibitor; PAS, plasminogen activation system; PHx, partial hepatectomy; Plg, plasminogen gene; TAFI, thrombin-activatable fibrinolysis inhibitor; tPA, tissue-type plasminogen activator; TXA, tranexamic acid; and uPA, urokinase-type plasminogen activator.

models is perhaps worthy of exploration. For example, what is the basis for the seemingly inconsistent impact of Plg deficiency on hepatic injury after APAP overdose? For 2 toxicants damaging the same zone of the liver (ie, CCl₄ and APAP), why is the impact of plasminogen deficiency on liver damage so different? If the key fibrinolytic protease plasminogen is proregenerative, how then is its primary substrate also proregenerative? Although these questions may ultimately have simple answers, the precise role of the PAS in liver injury and repair/regeneration is clearly not settled, and there is clearly a need for additional experiments to answer longstanding questions, particularly as new innovative *in vivo* tools are developed.

ARTICLE INFORMATION

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REFERENCES

- Risman RA, Kirby NC, Bannish BE, Hudson NE, Tutwiler VF. An illustrated review. *Res Pract Thromb Haemost*. 2023;7:100081. doi: 10.1016/j.rpth.2023.100081
- Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. *Nat Rev Mol Cell Biol*. 2010;11:23–36. doi: 10.1038/nrm2821
- Keragala CB, Medcalf RL. Plasminogen: an enigmatic zymogen. *Blood*. 2021;137:2881–2889. doi: 10.1182/blood.2020008951
- Lisman T, Bakhtiari K, Adelmeijer J, Meijers JC, Porte RJ, Stravitz RT. Intact thrombin generation and decreased fibrinolytic capacity in patients with acute liver injury or acute liver failure. *J Thromb Haemost*. 2012;10:1312–1319. doi: 10.1111/j.1538-7836.2012.04770.x
- Hughenoltz GC, Adelmeijer J, Meijers JC, Porte RJ, Stravitz RT, Lisman T. An unbalance between von Willebrand factor and ADAMTS13 in acute liver failure: implications for hemostasis and clinical outcome. *Hepatology*. 2013;58:752–761. doi: 10.1002/hep.26372
- Lisman T, Caldwell SH, Intagliata NM. Haemostatic alterations and management of haemostasis in patients with cirrhosis. *J Hepatol*. 2022;76:1291–1305. doi: 10.1016/j.jhep.2021.11.004
- Bleibel W, Caldwell SH, Curry MP, Northup PG. Peripheral platelet count correlates with liver atrophy and predicts long-term mortality on the liver transplant waiting list. *Transpl Int*. 2013;26:435–442. doi: 10.1111/tri.12064
- von Meijenfeldt FA, Lisman T. Fibrinolysis in patients with liver disease. *Semin Thromb Hemost*. 2021;47:601–609. doi: 10.1055/s-0040-1718924
- Maiwall R, Kulkarni AV, Arab JP, Piano S. Acute liver failure. *Lancet*. 2024;404:789–802. doi: 10.1016/S0140-6736(24)00693-7
- Henderson MW, Sparkenbaugh EM, Wang S, Ilich A, Noubouossie DF, Mailer R, Renne T, Flick MJ, Luyendyk JP, Chen ZL, et al. Plasmin-mediated cleavage of high-molecular-weight kininogen contributes to acetaminophen-induced acute liver failure. *Blood*. 2021;138:259–272. doi: 10.1182/blood.2020006198
- Driever EG, Stravitz RT, Zhang J, Adelmeijer J, Durkalski V, Lee WM, Lisman T. VWF/ADAMTS13 imbalance, but not global coagulation or fibrinolysis, is associated with outcome and bleeding in acute liver failure. *Hepatology*. 2021;73:1882–1891. doi: 10.1002/hep.31507
- Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. *J Hepatol*. 2023;79:516–537. doi: 10.1016/j.jhep.2023.03.017
- Lee WM. Acetaminophen-related acute liver failure in the United States. *Hepatol Res*. 2008;38:S3–S8. doi: 10.1111/j.1872-034X.2008.00419.x
- Borlak J, Chatterji B, Londhe KB, Watkins PB. Serum acute phase reactants hallmark healthy individuals at risk for acetaminophen-induced liver injury. *Genome Med*. 2013;5:86. doi: 10.1186/gm493
- Jaeschke H, McGill MR, Williams CD, Ramachandran A. Current issues with acetaminophen hepatotoxicity—a clinically relevant model to test the efficacy of natural products. *Life Sci*. 2011;88:737–745. doi: 10.1016/j.lfs.2011.01.025
- Ramachandran A, Jaeschke H. Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology. *J Clin Transl Res*. 2017;3:157–169. doi: 10.18053/jctres.03.2017S1.002
- Win S, Min RW, Chen CQ, Zhang J, Chen Y, Li M, Suzuki A, Abdelmalek MF, Wang Y, Aghajan M, et al. Expression of mitochondrial membrane-linked SAB determines severity of sex-dependent acute liver injury. *J Clin Invest*. 2019;129:5278–5293. doi: 10.1172/JCI128289
- Jaeschke H, Ramachandran A. Acetaminophen hepatotoxicity: paradigm for understanding mechanisms of drug-induced liver injury. *Annu Rev Pathol*. 2024;19:453–478. doi: 10.1146/annurev-pathmechdis-051122-094016
- Hinson JA, Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. *Handb Exp Pharmacol*. 2010;369:405. doi: 10.1007/978-3-642-00663-0_12
- Gao S, Silasi-Mansat R, Behar AR, Lupu F, Griffin CT. Excessive plasmin compromises hepatic sinusoidal vascular integrity after acetaminophen overdose. *Hepatology*. 2018;68:1991–2003. doi: 10.1002/hep.30070
- Sullivan BP, Kassel KM, Jone A, Flick MJ, Luyendyk JP. Fibrin(ogen)-independent role of plasminogen activators in acetaminophen-induced liver injury. *Am J Pathol*. 2012;180:2321–2329. doi: 10.1016/j.ajpath.2012.02.011
- Ganey PE, Luyendyk JP, Newport SW, Eagle TM, Maddox JF, Mackman N, Roth RA. Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice. *Hepatology*. 2007;46:1177–1186. doi: 10.1002/hep.21779
- Ben-Moshe S, Veg T, Manco R, Dan S, Papinutti D, Lifshitz A, Kolodziejczyk AA, Bahar Halpern K, Elinav E, Itzkovitz S. The spatiotemporal program of zonal liver regeneration following acute injury. *Cell Stem Cell*. 2022;29:973.e10–989.e10. doi: 10.1016/j.stem.2022.04.008
- Bajt ML, Yan HM, Farhood A, Jaeschke H. Plasminogen activator inhibitor-1 limits liver injury and facilitates regeneration after acetaminophen overdose. *Toxicol Sci*. 2008;104:419–427. doi: 10.1093/toxsci/kfn091
- Pant A, Kopec AK, Baker KS, Cline-Fedewa H, Lawrence DA, Luyendyk JP. Plasminogen activator inhibitor-1 reduces tissue-type plasminogen activator-dependent fibrinolysis and intrahepatic hemorrhage in experimental acetaminophen overdose. *Am J Pathol*. 2018;188:1204–1212. doi: 10.1016/j.ajpath.2018.01.010
- Hughenoltz GC, Meijers JC, Adelmeijer J, Porte RJ, Lisman T. TAFI deficiency promotes liver damage in murine models of liver failure through defective down-regulation of hepatic inflammation. *Thromb Haemost*. 2013;109:948–955. doi: 10.1160/TH12-12-0930
- Luyendyk JP, Schoenacker JG, Flick MJ. The multifaceted role of fibrinogen in tissue injury and inflammation. *Blood*. 2019;133:511–520. doi: 10.1182/blood-2018-07-818211
- Kopec AK, Joshi N, Cline-Fedewa H, Wojcicki AV, Ray JL, Sullivan BP, Froehlich JE, Johnson BF, Flick MJ, Luyendyk JP. Fibrin(ogen) drives repair after acetaminophen-induced liver injury via leukocyte αMβ2 integrin-dependent upregulation of Mmp12. *J Hepatol*. 2017;66:787–797. doi: 10.1016/j.jhep.2016.12.004
- Groeneveld DJ, Poole LG, Bouck EG, Schulte A, Wei Z, Williams KJ, Watson VE, Lisman T, Wolberg AS, Luyendyk JP. Robust coagulation activation and coagulopathy in mice with experimental acetaminophen-induced liver failure. *J Thromb Haemost*. 2023;21:2430–2440. doi: 10.1016/j.jtha.2023.03.040
- Capece GE, Luyendyk JP, Poole LG. Fibrinolysis-mediated pathways in acute liver injury. *Semin Thromb Hemost*. 2024;50:638–647. doi: 10.1055/s-0044-1779738

31. McGill MR, Jaeschke H. Animal models of drug-induced liver injury. *Biochim Biophys Acta Mol Basis Dis.* 2019;1865:1031–1039. doi: 10.1016/j.bbdis.2018.08.037
32. Tsuchida T, Lee YA, Fujiwara N, Ybanez M, Allen B, Martins S, Fiel MI, Goossens N, Chou HI, Hoshida Y, et al. A simple diet- and chemical-induced murine NASH model with rapid progression of steatohepatitis, fibrosis and liver cancer. *J Hepatol.* 2018;69:385–395. doi: 10.1016/j.jhep.2018.03.011
33. Slater TF, Cheeseman KH, Ingold KU. Carbon tetrachloride toxicity as a model for studying free-radical mediated liver injury. *Philos Trans R Soc Lond B Biol Sci.* 1985;311:633–645. doi: 10.1098/rstb.1985.0169
34. von Montfort C, Beier JJ, Kaiser JP, Guo L, Joshi-Barve S, Pritchard MT, States JC, Arteel GE. PAI-1 plays a protective role in CCl₄-induced hepatic fibrosis in mice: role of hepatocyte division. *Am J Physiol Gastrointest Liver Physiol.* 2010;298:G657–G666. doi: 10.1152/ajpgi.00107.2009
35. Kang LI, Isse K, Koral K, Bowen WC, Muratoglu S, Strickland DK, Michalopoulos GK, Mars WM. Tissue-type plasminogen activator suppresses activated stellate cells through low-density lipoprotein receptor-related protein 1. *Lab Invest.* 2015;95:1117–1129. doi: 10.1038/labinvest.2015.94
36. Pohl JF, Melin-Aldana H, Sabla G, Degen JL, Bezerra JA. Plasminogen deficiency leads to impaired lobular reorganization and matrix accumulation after chronic liver injury. *Am J Pathol.* 2001;159:2179–2186. doi: 10.1016/S0002-9440(10)63069-6
37. Luyendyk JP, Mattes WB, Burgoon LD, Zacharewski TR, Maddox JF, Cosma GN, Ganey PE, Roth RA. Gene expression analysis points to hemostasis in livers of rats cotreated with lipopolysaccharide and ranitidine. *Toxicol Sci.* 2004;80:203–213. doi: 10.1093/toxsci/kfh146
38. Shaw PJ, Fullerton AM, Scott MA, Ganey PE, Roth RA. The role of the hemostatic system in murine liver injury induced by coexposure to lipopolysaccharide and trovafloxacin, a drug with idiosyncratic liability. *Toxicol Appl Pharmacol.* 2009;236:293–300. doi: 10.1016/j.taap.2009.01.018
39. Wang H, Vohra BP, Zhang Y, Heuckeroth RO. Transcriptional profiling after bile duct ligation identifies PAI-1 as a contributor to cholestatic injury in mice. *Hepatology.* 2005;42:1099–1108. doi: 10.1002/hep.20903
40. Wang H, Zhang Y, Heuckeroth RO. Tissue-type plasminogen activator deficiency exacerbates cholestatic liver injury in mice. *Hepatology.* 2007;45:1527–1537. doi: 10.1002/hep.21613
41. Wang H, Zhang Y, Heuckeroth RO. PAI-1 deficiency reduces liver fibrosis after bile duct ligation in mice through activation of tPA. *FEBS Lett.* 2007;581:3098–3104. doi: 10.1016/j.febslet.2007.05.049
42. Berghem I, Guo L, Davis MA, Duveau I, Arteel GE. Critical role of plasminogen activator inhibitor-1 in cholestatic liver injury and fibrosis. *J Pharmacol Exp Ther.* 2006;316:592–600. doi: 10.1124/jpet.105.095042
43. Bezerra JA, Currier AR, Melin-Aldana H, Sabla G, Bugge TH, Kombrinck KW, Degen JL. Plasminogen activators direct reorganization of the liver lobule after acute injury. *Am J Pathol.* 2001;158:921–929. doi: 10.1016/S0002-9440(10)64039-4
44. Bezerra JA, Bugge TH, Melin-Aldana H, Sabla G, Kombrinck KW, Witte DP, Degen JL. Plasminogen deficiency leads to impaired remodeling after a toxic injury to the liver. *Proc Natl Acad Sci U S A.* 1999;96:15143–15148. doi: 10.1073/pnas.96.26.15143
45. Roth K, Strickland J, Joshi N, Deng M, Kennedy RC, Rockwell CE, Luyendyk JP, Billiar TR, Copple BL. Dichotomous role of plasmin in regulation of macrophage function after acetaminophen overdose. *Am J Pathol.* 2019;189:1986–2001. doi: 10.1016/j.ajpath.2019.07.003
46. Shanmukhappa K, Matte U, Degen JL, Bezerra JA. Plasmin-mediated proteolysis is required for hepatocyte growth factor activation during liver repair. *J Biol Chem.* 2009;284:12917–12923. doi: 10.1074/jbc.M807313200
47. Roth K, Strickland J, Copple BL. Regulation of macrophage activation in the liver after acute injury: role of the fibrinolytic system. *World J Gastroenterol.* 2020;26:1879–1887. doi: 10.3748/wjg.v26.i16.1879
48. Ma X, Huang T, Chen X, Li Q, Liao M, Fu L, Huang J, Yuan K, Wang Z, Zeng Y. Molecular mechanisms in liver repair and regeneration: from physiology to therapeutics. *Signal Transduct Target Ther.* 2025;10:63. doi: 10.1038/s41392-024-02104-8
49. European Association for the Study of the Liver. EASL clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol.* 2018;69:182–236. doi: 10.1016/j.jhep.2018.03.019
50. Mendizabal M, Silva MO. Liver transplantation in acute liver failure: a challenging scenario. *World J Gastroenterol.* 2016;22:1523–1531. doi: 10.3748/wjg.v22.i4.1523
51. Nevzorova YA, Tolba R, Trautwein C, Liedtke C. Partial hepatectomy in mice. *Lab Anim.* 2015;49:81–88. doi: 10.1177/0023677215572000
52. Michalopoulos GK, Bhushan B. Liver regeneration: biological and pathological mechanisms and implications. *Nat Rev Gastroenterol Hepatol.* 2021;18:40–55. doi: 10.1038/s41575-020-0342-4
53. Mars WM, Liu ML, Kitson RP, Goldfarb RH, Gabauer MK, Michalopoulos GK. Immediate early detection of urokinase receptor after partial hepatectomy and its implications for initiation of liver regeneration. *Hepatology.* 1995;21:1695–1701.
54. Mars WM, Kim TH, Stolz DB, Liu ML, Michalopoulos GK. Presence of urokinase in serum-free primary rat hepatocyte cultures and its role in activating hepatocyte growth factor. *Cancer Res.* 1996;56:2837–2843.
55. Lieber A, Vrancken Peeters MJ, Meuse L, Fausto N, Perkins J, Kay MA. Adenovirus-mediated urokinase gene transfer induces liver regeneration and allows for efficient retrovirus transduction of hepatocytes in vivo. *Proc Natl Acad Sci U S A.* 1995;92:6210–6214. doi: 10.1073/pnas.92.13.6210
56. Roselli HT, Su M, Washington K, Kerins DM, Vaughan DE, Russell WE. Liver regeneration is transiently impaired in urokinase-deficient mice. *Am J Physiol.* 1998;275:G1472–G1479. doi: 10.1152/ajpgi.1998.275.6.G1472
57. Nomura K, Miyagawa S, Ayukawa K, Soeda J, Taniguchi S, Kawasaki S. Inhibition of urokinase-type plasminogen activator delays expression of c-jun, activated transforming growth factor beta 1, and matrix metalloproteinase 2 during post-hepatectomy liver regeneration in mice. *J Hepatol.* 2002;36:637–644. doi: 10.1016/S0168-8278(02)00024-7
58. Mars WM, Zarnegar R, Michalopoulos GK. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. *Am J Pathol.* 1993;143:949–958.
59. Nishimura K, Matsumiya K, Miura H, Tsujimura A, Nonomura N, Matsumoto K, Nakamura T, Okuyama A. Effects of hepatocyte growth factor on urokinase-type plasminogen activator (uPA) and uPA receptor in DU145 prostate cancer cells. *Int J Androl.* 2003;26:175–179. doi: 10.1046/j.1365-2605.2003.00413.x
60. Lee KH, Choi EY, Kim MK, Hyun MS, Jang BI, Kim TN, Kim SW, Song SK, Kim JH, Kim JR. Regulation of hepatocyte growth factor-mediated urokinase plasminogen activator secretion by MEK/ERK activation in human stomach cancer cell lines. *Exp Mol Med.* 2006;38:27–35. doi: 10.1038/emmm.2006.4
61. Drixler T, Vogten JM, Gebbink MF, Carmeliet P, Voest EE, Borel Rinkes IH. Plasminogen mediates liver regeneration and angiogenesis after experimental partial hepatectomy. *Br J Surg.* 2003;90:1384–1390. doi: 10.1002/bjs.4275
62. Akao M, Hasebe Y, Okumura N, Hagiwara H, Seki T, Ariga T. Plasminogen activator-plasmin system potentiates the proliferation of hepatocytes in primary culture. *Thromb Res.* 2002;107:169–174. doi: 10.1016/S0049-3848(02)00258-X
63. Watanabe K, Togo S, Takahashi T, Matsuyama R, Yamamoto H, Shimizu T, Makino H, Matsuo K, Morioka D, Kubota T, et al. PAI-1 plays an important role in liver failure after excessive hepatectomy in the rat. *J Surg Res.* 2007;143:13–19. doi: 10.1016/j.jss.2007.04.041
64. Groeneveld D, Pereyra D, Veldhuis Z, Adelmeijer J, Ottens P, Kopec AK, Starlinger P, Lisman T, Luyendyk JP. Intrahepatic fibrin(ogen) deposition drives liver regeneration after partial hepatectomy in mice and humans. *Blood.* 2019;133:1245–1256. doi: 10.1182/blood-2018-08-869057
65. Moon AM, Singal AG, Tapper EB. Contemporary epidemiology of chronic liver disease and cirrhosis. *Clin Gastroenterol Hepatol.* 2020;18:2650–2666. doi: 10.1016/j.cgh.2019.07.060
66. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol.* 2017;14:397–411. doi: 10.1038/nrgastro.2017.38
67. Morrow GB, Mutch NJ. Past, present, and future perspectives of plasminogen activator inhibitor 1 (PAI-1). *Semin Thromb Hemost.* 2023;49:305–313. doi: 10.1055/s-0042-1758791
68. Schafer K, Fujisawa K, Konstantinides S, Loskutov DJ. Disruption of the plasminogen activator inhibitor 1 gene reduces the adiposity and improves the metabolic profile of genetically obese and diabetic ob/ob mice. *FASEB J.* 2001;15:1840–1842. doi: 10.1096/fj.00-0750fje
69. Piao L, Jung I, Huh JY, Miyata T, Ha H. A novel plasminogen activator inhibitor-1 inhibitor, TM5441, protects against high-fat diet-induced obesity and adipocyte injury in mice. *Br J Pharmacol.* 2016;173:2622–2632. doi: 10.1111/bph.13541
70. Lee SM, Drotea D, Jung I, Nakabayashi T, Miyata T, Ha H. TM5441, a plasminogen activator inhibitor-1 inhibitor, protects against high fat diet-induced non-alcoholic fatty liver disease. *Oncotarget.* 2017;8:89746–89760. doi: 10.18632/oncotarget.21120
71. Henkel AS, Khan SS, Olivares S, Miyata T, Vaughan DE. Inhibition of plasminogen activator inhibitor 1 attenuates hepatic steatosis but does not prevent progressive nonalcoholic steatohepatitis in mice. *Hepatol Commun.* 2018;2:1479–1492. doi: 10.1002/hep4.1259
72. Kim HY, Rosenthal SB, Liu X, Miciano C, Hou X, Miller M, Buchanan J, Poirion OB, Chilin-Fuentes D, Han C, et al. Multi-modal analysis of human hepatic stellate cells identifies novel therapeutic targets for metabolic

dysfunction-associated steatotic liver disease. *J Hepatol*. 2025;82:882–897. doi: 10.1016/j.jhep.2024.10.044

73. Tomonari Y, Iwaki T, Arakawa T, Umemura K. Inhibition of plasminogen suppresses fibrosis and macrophage foaming in a nonalcoholic steatohepatitis mouse model. *Fundam Clin Pharmacol*. 2022;36:827–836. doi: 10.1111/fcp.12774
74. Dai W, Castleberry M, Zheng Z. Tale of two systems: the intertwining duality of fibrinolysis and lipoprotein metabolism. *J Thromb Haemost*. 2023;21:2679–2696. doi: 10.1016/j.jtha.2023.08.004
75. Beier JI, Arteel GE. Alcoholic liver disease and the potential role of plasminogen activator inhibitor-1 and fibrin metabolism. *Exp Biol Med (Maywood)*. 2012;237:1–9. doi: 10.1258/ebm.2011.011255
76. Beier JI, Luyendyk JP, Guo L, von Montfort C, Staunton DE, Arteel GE. Fibrin accumulation plays a critical role in the sensitization to lipopolysaccharide-induced liver injury caused by ethanol in mice. *Hepatology*. 2009;49:1545–1553. doi: 10.1002/hep.22847
77. Bergheim I, Guo L, Davis MA, Lambert JC, Beier JI, Duveau I, Luyendyk JP, Roth RA, Arteel GE. Metformin prevents alcohol-induced liver injury in the mouse: critical role of plasminogen activator inhibitor-1. *Gastroenterology*. 2006;130:2099–2112. doi: 10.1053/j.gastro.2006.03.020
78. Liu X, Rosenthal SB, Meshgin N, Baglieri J, Musallam SG, Diggle K, Lam K, Wu R, Pan SQ, Chen Y, et al. Primary alcohol-activated human and mouse hepatic stellate cells share similarities in gene-expression profiles. *Hepatol Commun*. 2020;4:606–626. doi: 10.1002/hep4.1483
79. Hsiao Y, Zou T, Ling CC, Hu H, Tao XM, Song HY. Disruption of tissue-type plasminogen activator gene in mice aggravated liver fibrosis. *J Gastroenterol Hepatol*. 2008;23:e258–e264. doi: 10.1111/j.1440-1746.2007.05100.x
80. Leyland H, Gentry J, Arthur MJ, Benyon RC. The plasminogen-activating system in hepatic stellate cells. *Hepatology*. 1996;24:1172–1178. doi: 10.1002/hep.510240532
81. Joshi N, Kopec AK, Towery K, Williams KJ, Luyendyk JP. The antifibrinolytic drug tranexamic acid reduces liver injury and fibrosis in a mouse model of chronic bile duct injury. *J Pharmacol Exp Ther*. 2014;349:383–392. doi: 10.1124/jpet.113.210880
82. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet*. 2012;379:1245–1255. doi: 10.1016/S0140-6736(11)61347-0
83. Qiu S, Cai J, Yang Z, He X, Xing Z, Zu J, Xie E, Henry L, Chong CR, John EM, et al. Trends in hepatocellular carcinoma mortality rates in the US and projections through 2040. *JAMA Netw Open*. 2024;7:e2445525. doi: 10.1001/jamanetworkopen.2024.45525
84. Chen S, Morine Y, Tokuda K, Yamada S, Saito Y, Nishi M, Ikemoto T, Shimada M. Cancer-associated fibroblast-induced M2-polarized macrophages promote hepatocellular carcinoma progression via the plasminogen activator inhibitor-1 pathway. *Int J Oncol*. 2021;59:59. doi: 10.3892/ijo.2021.5239
85. Jin Y, Liang ZY, Zhou WX, Zhou L. Expression, clinicopathologic and prognostic significance of plasminogen activator inhibitor 1 in hepatocellular carcinoma. *Cancer Biomark*. 2020;27:285–293. doi: 10.3233/CBM-190560
86. Wei L, Lun Y, Zhou X, He S, Gao L, Liu Y, He Z, Li B, Wang C. Novel urokinase-plasminogen activator inhibitor SPINK13 inhibits growth and metastasis of hepatocellular carcinoma in vivo. *Pharmacol Res*. 2019;143:73–85. doi: 10.1016/j.phrs.2019.03.009
87. Bugge TH, Flick MJ, Daugherty CC, Degen JL. Plasminogen deficiency causes severe thrombosis but is compatible with development and reproduction. *Genes Dev*. 1995;9:794–807. doi: 10.1101/gad.9.7.794
88. Carmeliet P, Schoonjans L, Kieckens L, Ream B, Degen J, Bronson R, De Vos R, van den Oord JJ, Collen D, Mulligan RC. Physiological consequences of loss of plasminogen activator gene function in mice. *Nature*. 1994;368:419–424. doi: 10.1038/368419a0
89. Strilchuk AW, Hur WS, Batty P, Sang Y, Abrahams SR, Yong ASM, Leung J, Silva LM, Schroeder JA, Nesbitt K, et al. Lipid nanoparticles and siRNA targeting plasminogen provide lasting inhibition of fibrinolysis in mouse and dog models of hemophilia A. *Sci Transl Med*. 2024;16:eadh0027. doi: 10.1126/scitranslmed.adh0027