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REVIEW

Experimental Liver Surgery for Liver Research: Update, Choice and Translation

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Abstract: Experimental animal models of liver surgery are crucial for understanding human liver physiology and pathogenesis and identifying novel therapeutic modalities for liver disease. Herein, we update the brief summary of the most widely used experimental models and concepts in hepatic surgery, including hepatic ischemia/reperfusion, partial hepatectomy, liver transplantation, techniques and parameters of vascular perfusion of the liver, and using bile duct ligation as a model of cholestasis for the development of liver fibrosis. We focus on surgical aspects of available models for the study of various forms of liver disease. Furthermore, we summarize the translation of experimental liver surgery by highlighting surgical innovations, exploring key molecular mechanisms, and employing emerging treatment strategies.

Keywords: experimental liver surgery, hepatic ischemia/reperfusion, hepatectomy, liver transplantation, extracorporeal vascular perfusion, hepatic fibrosis

Background

Experimental animal models of liver surgery are crucial for basic research to study liver physiology and the molecular pathology of liver diseases in humans. The experimental models and concepts in hepatic surgery, including hepatic ischemia/reperfusion, partial hepatectomy, liver transplantation, techniques and parameters of vascular perfusion of the liver, and using bile duct ligation as a model of cholestasis for the development of liver fibrosis are commonly used for liver surgery and contribute to the understanding of liver physiology and pathogenesis of liver regeneration and inflammation, development and treatment of fibrosis, acute liver injury, acute liver failure, hepatic metastasis and tumor recurrence.[1–4]

Mice, rats, rabbits, dogs, pigs, and zebrafish are frequently used for the study of experimental models of liver surgery. Mice and rats are the most commonly used animals in liver surgical research because of their small size, rapid breeding, and easy handling; in addition, genetic homologous, availability of transgenic models, and the ability to explore the effects of biochemical and biological reagents allow the use of these small animal models to investigate specific pathways of disease that would otherwise not be possible in humans.

The establishment of experimental surgery models can be highly dependent on microsurgical techniques, which are particularly relevant for murine models and permit selective dissection and ligature of the hepatic vascular and biliary branches and liver resection. With advances in liver surgery, more sophisticated surgical techniques, including associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), have been developed in animal models.[5,6] It is necessary to update the animal models used for liver surgery. In addition, in current liver research, the use of experimental surgery in animal models has been expanded by emerging biological techniques, including the development of genetic editing methods such as gene chemistry, liver-targeted gene delivery systems, clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9),[7–9] and three-dimensional cell culture systems (spheroids, organoids, liver-on-a-chip systems), and the decellularization and repopulation of the human liver.[4,10] These emerging biological techniques allow a clear awareness of the role of experimental liver surgery in a particular new era of unprecedented progress in the biomedical sciences.

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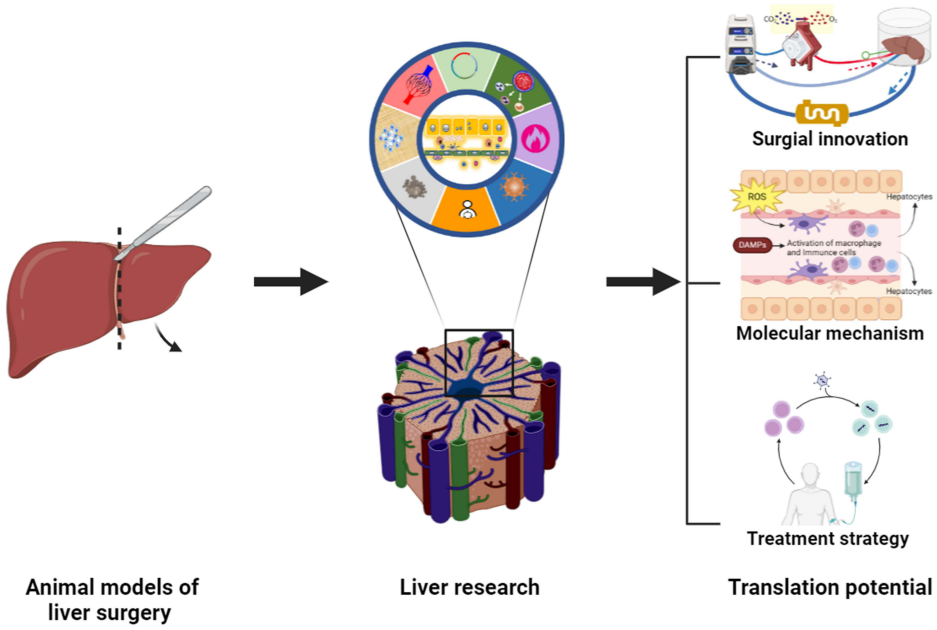
Graphical Abstract



Thus, in the current review, we summarize the widely used models of experimental surgery, focusing on surgical aspects, research applications, and translational value.

Animal Models of Liver Surgery

Hepatic Ischemia/Reperfusion

Hepatic ischemia/reperfusion injury inevitably occurs during liver surgery, which is evident by a rapid and marked increase in the serum levels of alanine aminotransferase and the proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), an accumulation of reactive oxygen species (ROS) and oxidative stress, and histopathologic damage, characterized by hemorrhage, hepatocellular necrosis, swelling of hepatocytes and cytoplasmic vacuolization, sinusoidal dilation and congestion in the centrilobular regions, and neutrophil infiltration throughout the necrotic areas and sinusoidal spaces.[1] Notably, hepatic ischemia/reperfusion triggers a time- and percentage-dependent increase in factors related to liver damage.[11]

Hepatic ischemia/reperfusion is usually classified into warm ischemia/reperfusion and cold ischemia/reperfusion; the former applies to the situation where vascular clamping inhibits hepatic blood supply followed by the restoration of blood flow, and the latter only applies to liver transplantation caused by cold-stored liver grafts prior to warm reperfusion.[1] An experimental model of hepatic warm ischemia/reperfusion is established by temporary occlusion of both the portal vein and its in-parallel artery simultaneously to hepatic segments of the liver or the entire liver in the whole or partial liver following the restoration of blood flow to the ischemic region.[12] The most commonly used experimental model of hepatic warm ischemia/reperfusion is induced by 70% hepatic ischemia for 60 min followed by reperfusion for 90 min-24 h in mice (Figure 1A and B). The model is simple, has a short modeling time, has a low cost, has high reproducibility, achieves an almost 100% survival rate, and requires short-term practice to master the surgical technique.[11]

Partial Hepatectomy

Partial hepatectomy in mice and rats is commonly used to study liver regeneration, acute liver failure, and tumor recurrence in response to liver injury. In 1931, Higgins and Anderson[13] created a standardized partial hepatectomy in rats

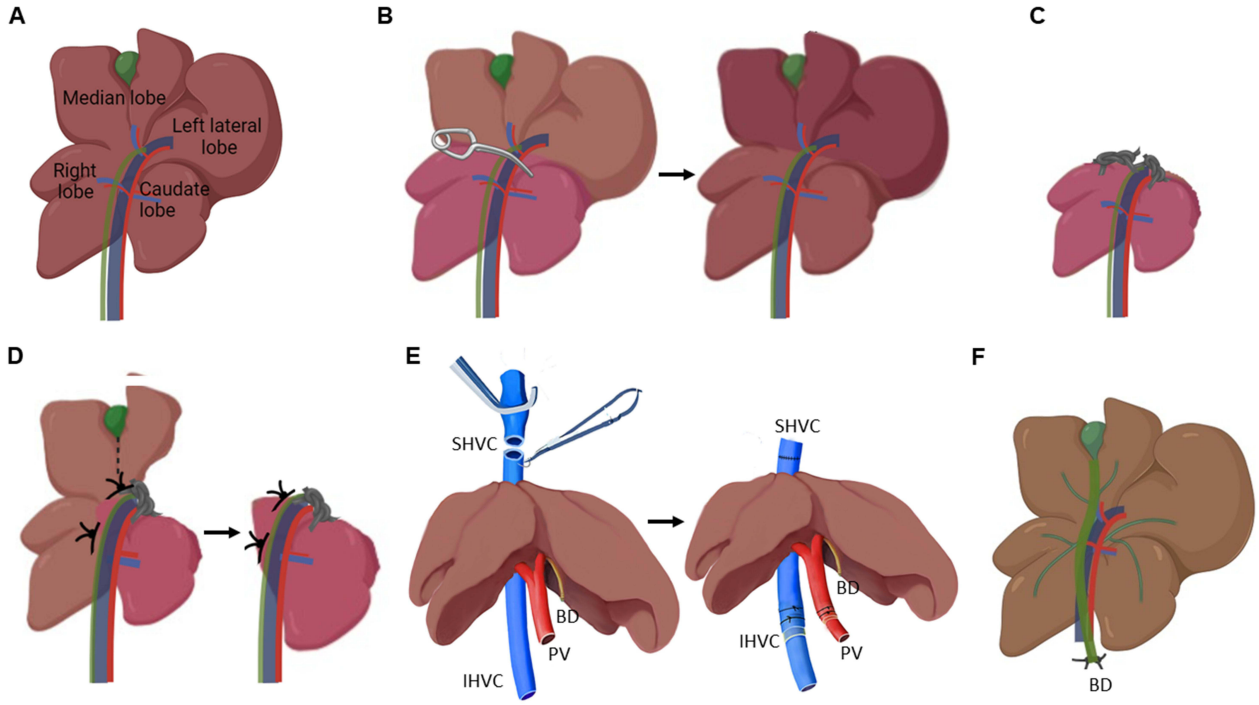


Figure 1 Induction of experimental liver surgery models. (**A**) A ventral view of the normal mouse liver with the left lateral lobe, median lobe, right lobe, and caudate lobe, with the portal vein (blue), hepatic artery (red) and common bile duct (green). (**B**) Seventy percent hepatic ischemia/reperfusion in mice: cross-clamping and restoring the portal vein and hepatic artery induces ischemia and reperfusion to the left lateral and median lobes of the liver, accounting for 70% of the liver parenchyma. (**C**) Seventy percent of partial hepatectomy in mice: resection of the median and left lateral lobes, which account for approximately 70% of the total liver. (**D**) Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) in rodents: in step 1 surgery, 90% portal vein ligation is combined with left lateral lobe (30% liver parenchyma) resection and transection between the median lobe; in step 2 surgery, the deportalized liver segments are removed, and later, the remnant caudate lobe (future liver remnant) shows significant hypertrophy. (**E**) Rat orthotopic liver transplantation: anastomosis of the suprahepatic inferior vena cava (SHVC), and anastomosis of the portal vein (PV) and infrahepatic inferior vena cava (IHVC) via the "two-cuff" technique with bile duct (BD) construction. (**F**) Bile duct ligation in mice: ligation of the common bile duct (BD, green). Created in Biorender. http://BioRender.com/60gsulv and http://Biorender.com/r8rv100.

through resection of the left lateral lobe and the median lobe, which accounts for approximately 70% of the total liver (Figure 1A and C). The classic 70% partial hepatectomy model in mice and rats is the most popular for liver regeneration research, because it can easily be accomplished with a single ligature *en bloc* at the base of the selected lobes and the animals can survive to study regeneration; 70–90% partial hepatectomy, which requires more ligations and the post-operative survival declines, is used to induce acute liver failure.[14] Partial hepatectomy in pigs represents the most suitable animal model for the study of liver regeneration because the size, anatomy and physiology of the pig liver are similar to those of the human liver.[6,15] Small animal models are less expensive and more suitable for basic research, while large animal models could provide more translational potential to humans.

ALPPS was first introduced in 2012 by Schnitzbauer et al[16] to improve the function and viability of the inadequate future liver remnant and overcome the occurrence of liver failure after hepatectomy. The ALPPS models in rodents (Figure 1D) and pigs were developed with 70–90% portal flow deprivation via selective portal vein ligation, parenchymal transection and partial hepatectomy (step 1), followed by resection of the liver with portal vein ligation (step 2),[5,6] which can reproduce pronounced liver regeneration comparable to that in humans during the ALPPS procedure.

Liver Transplantation

An animal model of liver transplantation provides an ideal research tool for studying the regulation of acute liver injury and transplant immunology.[17] Rat orthotopic liver transplantation has gained popularity as a preclinical liver transplantation model. Like clinical liver transplantation, the surgical procedure of a rat orthotopic liver transplantation model comprises three main stages: donor hepatectomy, back-table operation, and total hepatectomy and liver transplantation in

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the recipient. The rat is an ideal animal for the suitable size available for partial liver and arterial reconstruction, and the clear immunogenicity to induce a strong rejection.

In 1973, Lee et al[18] introduced the microsuture technique and successfully performed the first rat liver transplantation. Kamada and Calne[19] proposed the "two-cuff" technique to simplify the surgical procedure of rat liver transplantation, including the microsuture technique for anastomosing the suprahepatic inferior vena cava, the cuff technique for anastomosing the infrahepatic inferior vena cava and portal vein, and the splint technique for reconstruction of the bile duct without hepatic artery reconstruction (Figure 1E). Many modifications of the "two-cuff" technique have been applied in rats and mice, achieving high success rates and long survival time. However, there are still great challenges in the use of this model, including the difficulties of microsurgery operations, the prolonged surgery time, and the steep learning curve for surgeons.[20] Although liver transplantation in larger laboratory animals is technically easier, orthotopic rodent liver transplantation has still gained popularity because of the availability of genetically defined animals to study the molecular mechanism following surgical stress, and transplant immunology in the well-defined strains.

With the technical advances of auxiliary liver transplantation and ALPPS, partial liver transplantation and resection and partial liver segment II/III transplantation with delayed total hepatectomy (RAPID) in humans has been developed and is evolving.[21,22] However, animal models of auxiliary liver transplantation and RAPID are facing technical challenges, with results inferior to those of orthotopic liver transplantation. Currently, there is still no satisfactory animal model of auxiliary liver transplantation or RAPID. Thus, we use the ALPPS model of a staged hepatectomy[5] as an alternative model to study the concept and effects of the RAPID technique.[23]

Bile Duct Ligation

Bile duct ligation in mice and rats is a classic experimental model for the development of cholestasis and liver fibrosis because of its high reproducibility. This technique requires dissection and ligation of the bile duct to induce extrahepatic cholestasis (Figure 1F) and subsequent cholestasis-induced cirrhosis after 4–6 weeks, which is related to specific clinical conditions, including biliary atresia, choledocholithiasis and liver cirrhosis.[24] However, mortality due to bile leakage and gallbladder rupture that may occur during bile duct ligation is relatively high.[2]

Selection of the Liver Surgery Model for Liver Research

Acute Liver Injury

Acute liver injury in humans manifests as elevated levels of serum transaminases, decreased liver function, and pronounced hepatocyte death.[1] Liver injury can be divided into surgical-induced injury and drug-induced injury. Liver surgery, including hepatic ischemia/reperfusion and partial hepatectomy in mice, is a classic technique and a commonly used animal model for mimicking acute liver injury. It is believed that liver injury induced by liver surgery is initiated by surgical stress and ischemia/reperfusion injury, which cause direct hepatocyte injury and acute inflammatory changes. The other category is primarily caused by hepatotoxins such as 2-acetylaminofluorene (2-AAF) and carbon tetrachloride (CCl4). The combination of liver surgery and drug-induced hepatotoxicins could provide methods to explore liver injury and liver regeneration following liver failure.[1,3,25]

Acute Liver Failure

Acute hepatic failure occurs as a result of functional failure of the hepatic parenchyma, and its severity depends on the degree of liver injury. Partial hepatectomy and devascularization, as well as hepatotoxic drugs and viruses, are the major approaches used to create an animal model for acute hepatic failure. The 70–90% partial hepatectomy in mice and rats are used to study acute liver failure and to test the usefulness of artificial liver systems.[3,26] Devascularization of the liver can be achieved by simultaneous ligation of the partial portal vein and hepatic artery followed by a portacaval shunt in dog and pig models. Studies on the technical feasibility, safety, reproducibility and survival time of acute hepatic failure models have reported that partial hepatectomy is the most suitable technique for studying the status of reduced liver size, while devascularization is more suitable for studying the development of acute hepatic failure caused by ischemia.[26] The

use of a devascularization model for acute hepatic failure and portal hypertension has shown a decreasing trend over the past 10 years.

Liver Regeneration

The liver has a unique regenerative ability to restore its original mass and function following partial hepatectomy or toxic damage. Liver regeneration is an intricate biological process involving the proliferation of hepatocytes, as well as the activation and proliferation of nonparenchymal cells in the liver.[27,28] Various experimental models of liver injury in animals, including mice, rats, pigs, rabbits, and zebrafish, have been utilized to study liver regeneration, resulting in differing regenerative effects. The 70% hepatectomy model in mice and rats is still the most popular for studying liver regeneration. Following severe and prolonged liver injury, where the ability of hepatocytes to replicate is impaired, a second regenerative pathway marked by hepatic progenitor cells is activated. The hepatic progenitor cell-mediated liver regeneration has been investigated primarily in a rodent model of 70% hepatectomy combined with toxins (2-AAF or CCl4).[27]

Tumor Growth and Recurrence After Surgery

Xenografting of human liver cancer-derived cells or tumor tissue either into the liver or under the skin in immune-deficient mice is a well-established research tool in liver cancer research. The most common mouse strains used for xenografting are nude mice and severe combined immune-deficient mice. Recently, zebrafish have emerged as a host for xenograft of liver cancer cells and patient-derived liver cancer. Xenograft models that maintain key genetic features of their parental tumors are largely designed to study the molecular mechanism of tumor progression and tumor metabolism and test new drugs or combination therapies.[29]

In addition, experimental liver surgery models of hepatic ischemia/reperfusion, partial hepatectomy, and liver transplantation have been used to study liver metastasis and tumor recurrence after direct hepatic inoculation or after intraportal injection of rodent-derived tumor cells in immunocompetent rodents.[14,30] This model has been used to study the influence of hepatic ischemia/reperfusion injury and liver regeneration on liver cancer growth and metastasis, colorectal liver metastases, and the effect of adjuvant therapy on tumor growth.[31–33]

Transplantation Immunology

Acute Cellular Rejection

The animal model of liver transplantation is the foundation for research on transplantation rejection and tolerance. When liver transplantation in the animals uses the selected donor–recipient pairs, acute rejection has less severe manifestations or even no rejection. Rat orthotopic liver transplantation has gained popularity as an experimental liver transplantation model for transplantation immunology. The preferred rat strains selected for research on acute cellular rejection are LEW and BN as donors and recipients (LEW→BN) and DA→LEW; instead, reversing the strains of donors and recipients serves as a model for tolerance, that is, BN→LEW and LEW→DA. Like in rats, liver transplantation of C3H→B10 mice results in rejection, while B10→C3H shows a high tolerance rate.[34]

Xenotransplantation

Pigs are the most preferred candidate species for xenotransplantation in humans and monkeys because of their physiological similarity to primates and short maturation period. The applications of genetic editing methods and immunosuppressive therapy, such as strategies targeting hyperacute rejection and acute rejection, coagulation dysregulation and the inflammatory response, have improved xenograft survival in preclinical models, approaching the clinical application of xenotransplantation.[35,36] The deletion of identified xenoantigens, including galactose-α1,3-galactose, and the insertion of human complement regulatory proteins (CD46, CD55, or CD59), human coagulation regulatory proteins (thrombomodulin, endothelial cell protein C receptor), and human anti-inflammatory genes (CD47 and heme oxygenase-1) were engineered and detected efficiently in pigs as the humanized porcine donor. The genetically engineered porcine grafts could provide an successful approach for human transplantation and form the promising foundation for both preclinical and clinical trials.[35–37]

Translational Potential of Liver Research Based on Experimental Liver Surgery

The surgical interventions and findings in animals can largely contribute to humans, which are not always replicated or possible in human trials.[38] Herein we summarize the recognized surgical innovations, translational knowledge, and emerging treatments based on experimental liver surgery.

Surgical Innovations

It is necessary to address the value of microsurgical techniques in experimental models for liver research. Microsurgical techniques enable the establishment of liver surgery models and reduce surgical complications in animals, contributing to more standardized experimental models and knowledge translation.[39]

Ischemic Preconditioning to Ameliorate Acute Liver Injury

Interestingly, the application of brief periods of hepatic vascular occlusion can confer resistance to the deleterious effects of prolonged hepatic ischemia/reperfusion injury.[40] The beneficial effects of ischemic preconditioning demonstrated in experimental animal models of hepatic ischemia/reperfusion prompted further research in human trials. Intermittent Pringle maneuver, total occlusion of the portal arteries and vein corresponding to ischemic preconditioning in the clinic, has been successfully applied to prevent blood loss and reduce hepatic ischemia/reperfusion injury in clinical liver surgery, including human liver resection and liver transplantation.[11] Generally, a 5- to 10-minute period of ischemia followed by 10-minute reperfusion confers a hepatoprotective effect and is widely used as a routine practice.[41] Mechanistically, ischemic preconditioning can reduce energy requirements, decrease ROS and inflammation, maintain electrolyte and metabolic homeostasis, reduce hepatocyte apoptosis and improve microcirculatory perfusion in preconditioned livers through autophagy and endoplasmic reticulum stress.[42,43]

Liver Machine Perfusion to Decrease Cold Ischemia/Reperfusion Injury

Ex vivo mechanical vascular perfusion of the liver is an alternative method for preserving liver grafts for transplantation and has been developed to improve the use of extended-criteria donor allografts with hypothermic, subnormothermic, and normothermic machine perfusion systems.[44] Compared with hypothermic and subnormothermic perfusion, normothermic perfusion has a superior perfusion outcome in both clinical and preclinical models.[45] The normothermic ex vivo liver machine perfusion system has been successfully developed for liver transplantation and has been extensively studied in rats and pigs. Both the hepatic artery and portal vein are prepared for in situ cannulation, and livers are perfused with warmed, oxygenated perfusate to replicate physiological conditions and improve graft quality (Figure 2).[46,47] Beyond transplantation, machine perfusion could play an alternative role in extracorporeal liver support, cancer research, and pharmaceutical testing.

Exploring the Molecular Mechanism of Liver Regeneration and Tumor Recurrence

Liver Homeostasis and Liver Regeneration

The liver has a unique ability to maintain homeostasis and regenerate itself in response to injury, which has been known since the ancient Greek.[28] According to the Greek myth, Prometheus stole fire to mankind, and then was punished to eternal torment that his immortal liver was eaten by an eagle every day and re-grew every night. The myth of Prometheus becomes the symbol of liver regeneration in modern medicine. Since partial hepatectomy in rats was developed, liver regeneration has been well studied. The 70% hepatectomy model in the mouse and rat results in division and proliferation of hepatocytes, proceeding through initiation, proliferation, and termination phases. After liver resection, the remaining hepatocytes from the periportal vein to the pericenter zones quickly enter the cell cycle as compensatory proliferation, followed by the proliferation of nonparenchymal cells.[27,28]

With the development of lineage tracing technology to label specific hepatocyte subpopulations with zonation markers, liver zonation in homeostasis, liver injury and liver regeneration has been well described.[48–50] Hepatocytes in all zones, especially Zone 2 hepatocytes, contribute to homeostatic proliferation, while stem cells are not required.[48,51] When the liver is injured from liver surgery or hepatotoxicity, the regenerative process is initiated by hepatocyte proliferation or reprogramming.[52] In bile duct ligation- or hepatotoxicity (3,5-diethoxycarbonyl-1,4-dihydrocollidine or

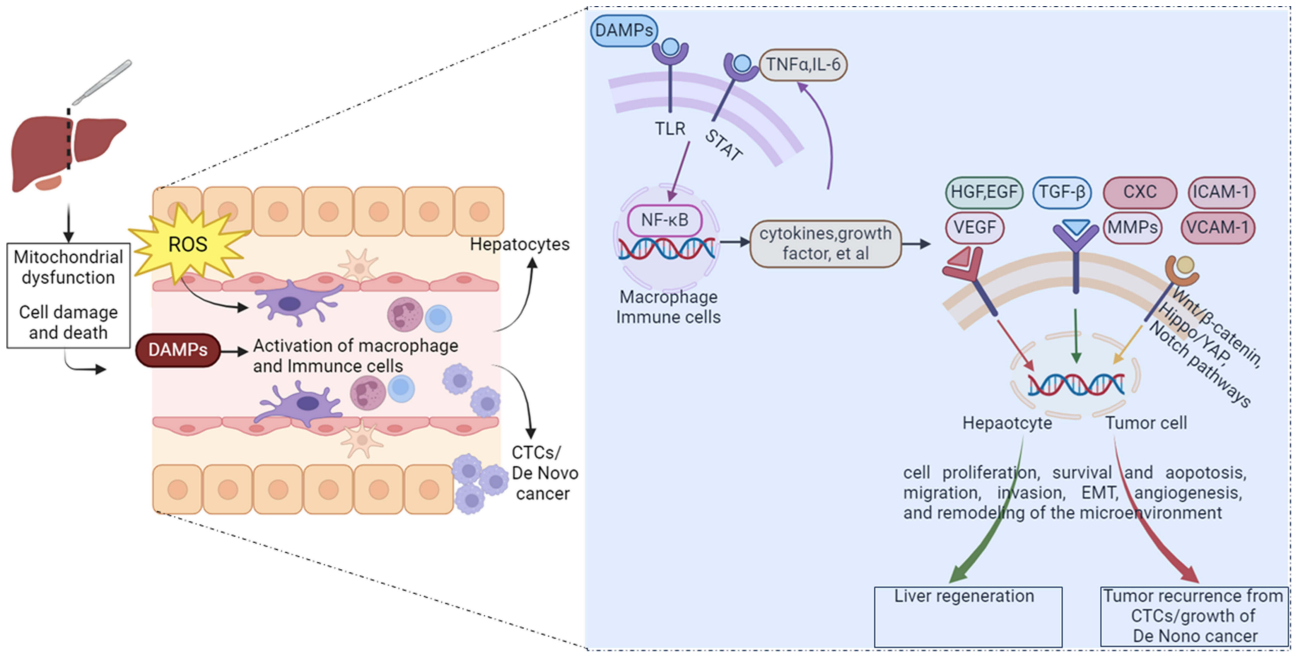

Figure 2 Contribution of liver injury to liver regeneration and tumor recurrence after liver surgery. Liver surgery causes liver injury and the activation of macrophages and immune cells through the reactive oxygen species (ROS) and damage associated molecular patterns (DAMPs), Toll-like receptor (TLR)/signal transducers and activation (STAT)-nuclear factor kappa B (NF-κB) pathways, leading to the release of ① proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6); ② growth factors, including hepatocyte growth factor (HGF), epidermal growth factor (EGF) and transforming growth factor-β (TGF-β); and ③ adhesion molecules, including intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), ④ C-X-C motif-type chemokines, ⑤ matrix-metalloproteases (MMPs), ⑥ vascular endothelial growth factor, and ⑦ the downstream Wnt/β-catenin, Hippo/YAP, and Notch pathways, which influence both liver regeneration and tumor recurrence from circulating tumor cells (CTCs). Following liver surgery, liver regeneration and tumor recurrence share the same mechanisms, including cell proliferation, survival and anti-apoptosis, migration, invasion, epithelial–mesenchymal transition (EMT), angiogenesis, and remodeling of the microenvironment. Created withBiorender.com. http://BioRender.com/e8tfot2 and http://BioRender.com/8929yqb.

allyl alcohol)-induced injury predominantly impacting Zone 1, hepatocytes in Zones 2 and 3 adjacent to the injured area proliferate to compensate for the increasing clone number and clone size in Zone 2.[48] In CCl4-induced injury impacting Zone 3, hepatocytes in Zones 1 and 2 adjacent to the injured area proliferate to compensate for the increase in clone number and clone size in Zone 2.[48] In the context of hepatocyte reprogramming, hepatocytes can be reprogrammed into either hepatic progenitor cells or biliary epithelial cells, subsequently contributing to approximately 25% of hepatocyte regeneration.[52] During hepatocyte proliferation and reprogramming, fine-tuning of IL-6/signal transducer and activator of transcription 3, Hippo/Yes-associated protein (YAP), Wnt/β-catenin, Hedgehog and TGF-β signaling in hepatocytes and cholangiocytes plays essential roles in maintaining liver metabolic function, liver size and regeneration, and the formation and repair of the bile duct, as highlighted by molecular mechanisms.[50,52–55]

Tumor Recurrence After Liver Surgery

Hepatectomy, liver transplantation, and local ablation are the three radical treatments for primary liver cancer and liver metastasis. However, liver surgery itself can activate occult liver cancer and accelerate *de novo* liver metastasis. The observation of this clinical phenomenon has been derived via study of animal models. Thus, the recurrence of primary and secondary liver cancer is a striking phenomenon after liver surgery.[31–33]

Acute liver injury following liver surgery causes cell damage and death, hemorrhage, ROS and oxidative stress, leading to the release of ① pathogen-associated molecular pattern molecules (PAMPs) and damage-associated molecular patterns (DAMPs); ② growth factors, including hepatocyte growth factor (HGF), epidermal growth factor (EGF), and transforming growth factor-β (TGF-β); ③ cytokines, including TNF-α, IL-6, IL-1β, interferon-β (INF-β), and INF-γ; ④ adhesion molecules, including E-selectin, intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1); ⑤ chemokines (CC-type and CXC-type); ⑥ matrix metalloproteases (MMPs); ⑦ inducible nitric oxide synthetase, and hypoxia inducible factor-1α activation; and ⑧ vascular endothelial growth factor.[50] Finally, downstream pathways, including the Hippo/YAP, Wnt/β-catenin, Notch, and Hedgehog pathways,[50,52–55] are triggered and contribute to tumor progression, epithelial-to-mesenchymal transition,

angiogenesis, and microenvironment remodeling, which favor tumor engraftment from circulating tumor cells and *de novo* cancer (Figure 2). Notably, tumorigenesis and perioperative liver injury share some same signaling pathways and have similar microenvironments; thus, perioperative liver injury may increase the occurrence of tumor metastasis and recurrence.[31–33] In addition, the growth of recurrent tumors is dependent on the size of the hepatectomy and the degree of liver regeneration.[30] Treatments for managing acute liver injury, including hypothermic machine perfusion and ischemia-free liver transplantation, have been shown to reduce tumor recurrence.[33,56,57]

Emerging Treatment Strategies Targeting Liver Injury

Pharmacological Therapy

During liver surgery, pharmacological interventions reportedly help mitigate liver injury and thereby confer hepatic protection.

1. Mitochondrial dysfunction initiates and exacerbates the acute liver injury cascade.[58,59] Experimental work in animal model has shown that novel pharmacological therapies targeting ROS, key inflammatory components, and the downstream inflammatory nuclear factor kappa B (NF-κB) and nuclear factor erythroid 2-related factor 2 (Nrf2)-heme oxygenase-1 (HO-1) signaling pathways in liver sinusoidal endothelial cells, Kupffer cells, stellate cells and immune cells, such as N-acetylcysteine and taurine, have been used to reduce hepatic ischemia/reperfusion injury and boost liver regeneration.[58–60]
2. Hemorheological factors are vital for maintaining endothelial functions and determining tissue perfusion. Nitric oxide agonists and endothelin antagonists, such as prostaglandin E1 (PGE1) and L-arginine, can improve hepatic blood flow and potently protect against hepatic ischemia/reperfusion injury.[61]
3. Targeting programmed cell death pathways, including apoptosis, pyroptosis and ferroptosis pathways, is a promising approach for ameliorating liver injury.[62–64] The major apoptotic markers including B-cell lymphoma-2 family proteins, apoptosis signaling pathways including JNK/p38 and PI3K/Akt signaling pathways, and the epigenetic modifications such as acetylation/deacetylation and ubiquitination/ deubiquitination of histone contribute to apoptosis, and targeting the apoptotic markers and signaling pathways can regulate liver ischemia-reperfusion injury.[62] Pyroptosis can be identified by sensor nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing protein 3 inflammasome, adaptor apoptosis-associated speck-like protein, effector inflammatory protease caspase-1,4,5,11, and the release of IL-1β and IL-18 cytokines and the cleaved gasdermin D. Inhibiting pyroptosis is indicated to improve liver ischemia-reperfusion injury by inhibiting the release and function of the effector caspases during liver ischemia-reperfusion injury.[63] Dysregulated iron metabolism and accumulation of lipid peroxides drive ferroptosis and trigger acute liver injury, especially through glutathione peroxidase 4/glutathione peroxidase 4 axis. Ferroptosis inhibitors such as ferrostatin-1 (Fer-1) can prevent ferroptosis and reduce liver ischemia-reperfusion injury.[64]

Nanozyme

Experimental work in multiple animal models have shown that, nanozymes, as a research frontier, have presented compelling evidence and exhibit great potential for constructing antioxidant nanozymes for preventing liver injury. Significantly increased ROS accumulation and inflammatory responses are crucial for the development of acute liver injury. As mimics of oxidoreductases and hydrolases, antioxidant nanozymes with simulated catalase and superoxide dismutase activity and hydroxyl radical antioxidant capacity can eliminate excess ROS in a catalytic manner to ameliorate the inflammatory microenvironment, thereby offering an appealing therapy for ameliorating acute liver injury. The available ROS-scavenging nanozymes include noble metals, metal oxides, carbon-based nanomaterials, and metal-organic frameworks.[65,66] More efforts are focused on improving the catalytic efficiency and targeted modification of nanozymes.

Gene Therapy

Gene therapy can mitigate hepatic ischemia/reperfusion injury or counteract acute and chronic graft rejection through the introduction or modulation of the respective genes. The biochemical approaches used for gene therapy include the use of antisense oligonucleotides or antagomirs to introduce or modify messenger RNA and the use of RNA molecules such as

microRNAs, short hairpin RNAs, or small interfering RNAs. The introduction of the cytoprotective genes heme oxygenase-1 and nuclear erythroid 2-related factor 2, the delivery of superoxide dismutase or IL-10 genes, and RNA interference against proteins such as high mobility group box 1, TNF-α, caspase-3/8, RelB (NF-κB subunit), and TNF receptor-associated factor have successfully attenuated experimental hepatic ischemia/reperfusion injury. All these findings have been possible through the use of many animal models. However, clinical translation is limited by vector design, safety, and gene delivery.[7,8]

Mesenchymal Stem Cell Transplantation

Stem cell therapy, particularly mesenchymal stem cell therapy, exerts anti-inflammatory and regenerative effects. Mesenchymal stem cell transplantation through intraportal injection is recognized as a promising cell therapy for liver failure in experimental models of liver surgery combined with drug-induced hepatotoxicins (2-AAF or CCl4). The hepatoprotective mechanism of mesenchymal stem cells is mediated by the modulation of adaptive and innate immune responses through the secretion of human leukocyte antigen-G1 (HLA-G1), HLA-G5, HGF, TGF-β1, IL-6, IL-10, C-C motif chemokine ligand 2 (CCL2), CCL7, TNF-stimulated gene-6, indoleamine 2.3-dioxygenase, and PGE2 and direct cell-to-cell interactions with membrane-bound molecules.[67]

Bioengineering

Decellularized porcine liver has been widely used in liver research, including developing humanized livers, and studying intrahepatic scaffold, angiogenic substances and liver-specific matrix substrates.[10] Furthermore, bioengineering approaches using decellularized liver scaffolds and liver-on-a-chip and 3D printing scaffolds to generate fully functional liver tissue ex vivo have been proposed as promising alternatives for replacing the liver, reproducing the liver microstructure and maintaining hepatic function.[10] Currently, bioengineering techniques, including the use of stem cells, senolytics, or molecules targeting mitochondria or downstream signaling during liver machine perfusion, are designed to modulate liver repair and regeneration mechanisms and improve the quality of liver grafts.[4]

Limitations and Summary

Although experimental animal models of liver surgery are the best tools for studying the physiology and pathology of the liver and developing new treatment strategies, translating these findings are usually challenging. Among the primary obstacles are differences in the anatomy, function and metabolism of the livers of various species. Furthermore, it is essential to choose the animal species and liver surgery model and to standardize the protocol. Mice and rats are more useful because they are easy to manipulate, more cost-effective, and have the potential for genetic editing. However, a major drawback is that the research findings are limited in humans due to differences in liver anatomy and metabolism. For instance, mice and rats have a lobed liver, and the lobe-based hepatectomy is easily performed, whereas human liver has a non-lobed liver and hepatectomy inevitably leads to liver parenchymal transection. Given the similarities between pigs and humans in terms of liver anatomy and physiology, pigs are better for studying problems of direct clinical relevance, especially in xenotransplantation and liver machine perfusion. However, their use is restricted by financial difficulties and ethical concerns.

Conclusions

In summary, we updated the widely used animal models of liver surgery, focusing on surgical aspects and research choices. The bridging of the translation gap between preclinical modeling and human research has focused on the evolution of surgical innovation, mechanism exploration, and emerging treatment strategies and is pushing toward clinical application. In the future, advances in experimental liver surgery may provide a foundation for scientific contributions to medical and biotechnological innovations.

Abbreviations

2-AAF, 2-acetylaminofluenorene; IL-1β, interleukin-1β; ALT, alanine aminotransferase; IL-6, interleukin-6; ALPPS, associating liver partition and portal vein ligation for staged hepatectomy; RAPID, resection and partial liver segment II/

III transplantation with delayed total hepatectomy; CCl4, carbon tetrachloride; MMP, matrix metalloprotease; CCL2, C-C motif chemokine ligand 2, ROS, reactive oxygen species; CRISPR/Cas9, clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated 9; TNF-α, tumor necrosis factor alpha; DAMP, damage associated molecular pattern; NF-κB, nuclear factor kappa B; EGF, epidermal growth factor; PAMP, pathogen-associated molecular pattern molecule; HGF, hepatocyte growth factor; PGE1, prostaglandin E1; HLA-G1, human leukocyte antigen-G1; PGE2, prostaglandin E2; ICAM-1, intercellular cell adhesion molecule-1; TGF-β, transforming growth factor-β; INF-β, interferon-β; VCAM-1, vascular cell adhesion molecule-1; INF-γ, interferon-γ; YAP, Yes-associate protein.

Data Sharing Statement

Data and material are available upon reasonable request from the corresponding authors.

Consent for Publication

All the authors declare that the work has not been published before, and consent the publication after the acceptance by the publication.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no financial interests or personal relationships that may influence the work reported in this paper.

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