

The role of ischaemia-reperfusion injury and liver regeneration in hepatic tumour recurrence



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Summary

The risk of cancer recurrence after liver surgery mainly depends on tumour biology, but preclinical and clinical evidence suggests that the degree of perioperative liver injury plays a role in creating a favourable microenvironment for tumour cell engraftment or proliferation of dormant micro-metastases. Understanding the contribution of perioperative liver injury to tumour recurrence is imperative, as these pathways are potentially actionable. In this review, we examine the key mechanisms of perioperative liver injury, which comprise mechanical handling and surgical stress, ischaemia-reperfusion injury, and parenchymal loss leading to liver regeneration. We explore how these processes can trigger downstream cascades leading to the activation of the immune system and the pro-inflammatory response, cellular proliferation, angiogenesis, anti-apoptotic signals, and release of circulating tumour cells. Finally, we discuss the novel therapies under investigation to decrease ischaemia-reperfusion injury and increase regeneration after liver surgery, including pharmaceutical agents, inflow modulation, and machine perfusion.

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Introduction

With a higher number of patients with new and more extensive malignancies accepted for liver transplantation, the field is currently focused on the implementation of novel interventions and surgical techniques to expand the pool of usable donor livers. However, a better understanding of key mechanisms is essential for the successful treatment of such complex patients and to avoid the most feared complications of graft loss, tumour recurrence and patient death.

Tumour recurrence after surgery is a common occurrence for both primary and secondary liver tumours, including hepatocellular carcinoma (HCC),¹ cholangiocarcinoma,² neuroendocrine tumours³ and colorectal liver metastases (CRLMs).^{4,5} Recurrence occurs in up to 70% of cases after liver resection, with the liver being the most common site.^{6–9} Liver transplantation for oncological indications is also burdened by significant post-transplant recurrence, up to 15% for HCC¹⁰ and over 80% for CRLMs.^{11,12} Unlike for resection, post-transplant recurrence is most commonly extrahepatic.^{7,13,14}

Despite the different tumour recurrence patterns, the mechanisms and risk factors are similar and multifactorial. A major role is certainly played by the tumour *per se*, with risk factors including size and number of lesions,^{15,16} tumour markers (alpha-feto-protein,¹⁵ carcinoembryonic antigen,¹⁷ carbohydrate antigen 19-9), degree of differentiation¹⁶ and vascular

invasion.¹⁸ However, intraoperative and early post-operative risk factors are increasingly recognized.

For transplantation, these risk factors can be related to donor characteristics and liver quality^{19,20} (older age, degree of steatosis, cold ischaemia time [CIT],^{21,22} donation after cardiac death [DCD]), or to recipient risk factors (obesity,²³ viral aetiology of underlying liver disease²⁴), and post-transplant immunosuppression.²⁵ For resections, a higher degree of liver manipulation during surgery has been linked to an increase in tumour recurrence and impaired liver regeneration, which involves various multifactorial pathways. For example, patient-based factors (*i.e.*, age, comorbidities,²⁶ previous chemotherapy²⁷), liver parenchyma quality (*i.e.*, cirrhosis,²⁸ fibrosis, cholestasis, and steatosis^{29,30}), and the resection procedure itself (extent of liver resection, blood loss, ischaemic damage) were all described to contribute to delayed liver regeneration and repair.

Both transplantation and resection require a certain degree of postoperative liver regeneration to avoid small for size syndrome and post-hepatectomy liver failure.³¹ Recently, evidence has emerged that tumour cell engraftment is also triggered by mechanisms linked to the process of liver resection and transplantation itself, with mitochondria being fundamental in both processes and key to immediate liver and patient recovery.

The link between mitochondrial alterations and cancer development has long been recognized.³²

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While it is still to be determined whether those alterations have a causal effect or are consequences of the carcinogenic process, it is established that mitochondria are essential for the survival, proliferation, and invasiveness of cancer cells through pathways related to energy production, survival in the stressful tumoral microenvironment, and avoidance of cell death. Similarly, hepatectomy and liver transplantation, by causing cellular damage through parenchymal transection, perfusion alterations and ischaemia-reperfusion injury (IRI), trigger a compensatory mitochondrial response in hepatocytes and other liver cells. This response leads to the activation of downstream pro-inflammatory cascades with consequences both at the local and systemic level, though the full spectrum of underlying mechanisms has yet to be completely appreciated.

In this review, we will discuss the main pathways involved in ischaemia-reperfusion injury and liver regeneration, and describe how these processes are ultimately linked to cancer cell engraftment and tumour recurrence. We will also summarize the novel therapies which are under investigation to target these mechanisms, as well as possible future directions for research.

Liver regeneration

The liver has a remarkable capacity for regeneration: depending on parenchymal quality, up to 75% of liver volume can be resected without inducing post-hepatectomy liver failure. This feature is at the basis of liver resection, as well as a requirement to avoid small for size syndrome after liver transplantation with small grafts, splits, and living donor liver transplant(ation) (LDLT). Advanced techniques of hypertrophy induction have been developed to enable hepatectomy in patients who would otherwise have an insufficient post-hepatectomy liver remnant volume, including portal vein embolization (PVE), hepatic vein embolization, two-staged hepatectomy (TSH), and associated liver partition and portal vein ligation for staged-hepatectomy (ALPPS). Recently, methods of auxiliary liver transplantation that exploit liver regeneration have been introduced, such as the RAPID³³ and RAVAS³⁴ technique. In these techniques, an auxiliary graft is implanted while maintaining the native liver completely or partially in place. Once the auxiliary graft has hypertrophied to a volume sufficient to sustain the metabolic needs of the body, a completion hepatectomy of the native liver is performed.

Liver regeneration implies an orderly sequence of events which starts 5 min after the start of parenchymal transection and shares many common pathways with wound healing, carcinogenesis, intrahepatic metastatic progression, and response to IRI. Indeed, some IRI is present at the start of liver regeneration.

Unlike at other sites, liver regeneration occurs through differentiation of fully differentiated cells for the most part,³⁵ with the support of oval cells (liver stem cells). As previously mentioned, mitochondria are essential for liver regeneration, which requires an enormous amount of energy production to support both liver hypertrophy and liver function.^{36,37} Mitochondrial bioenergetics appear to be directly correlated with postoperative liver function after hepatectomy as well as post-hepatectomy volumetric increase.³⁸

Factors that impair liver regeneration are similar to those increasing susceptibility to IRI (e.g. age, comorbidities, quality of the parenchyma), as well as factors related to the extent of

Key points

- Tumour recurrence occurs frequently after both liver resection and liver transplantation.
- While tumour biology and patient factors are major determinants of the risk of recurrence, perioperative factors such as mechanical handling, ischaemia-reperfusion injury and liver regeneration also play a role.
- These perioperative factors are responsible for the activation of downstream pathways that promote the release of circulating tumour cells and stimulate cell proliferation in a pro-inflammatory microenvironment that favours tumour engraftment.
- Mitochondrial dysfunction represents the starting point of the perioperative liver injury cascade.
- Novel therapies targeting mitochondria or downstream pathways are under investigation to reduce perioperative liver injury, including pharmaceutical agents, inflow modulation, and machine perfusion.
- Low quality evidence suggests that hypothermic oxygenated machine perfusion (HOPE) and ischaemia-free liver transplantation may reduce post-transplant cancer recurrence by diminishing perioperative liver injury.

resection and blood loss. The degree of IRI itself also influences the capacity to regenerate (i.e., the higher the IRI, the more difficult regeneration is going to be).

Liver regeneration can be divided into three phases: initiation (up to 5 h), proliferation (from 5 to 144 h), and termination.³⁹ Liver volume is usually restored in about a week in mice³⁵ and between 2 to 12 weeks in humans.³⁵

Initiation

After resection, liver volume diminishes while the portal venous flow remains unaltered. This leads to a sudden increase in portal venous pressure, which triggers initiation of liver regeneration. The increase in portal venous pressure causes shear stress and mechanical tension, as well as an increased concentration of growth factors, cytokines and liposaccharide (LPS) from the portal circulation. Shear stress activates urokinase plasminogen activator,⁴⁰ which upregulates matrix metalloproteinases (MMPs), causing matrix remodelling and activating hepatocyte growth factor (HGF).^{35,41}

During the first hour of the initiation phase, the Wnt/ β -catenin pathway is activated.^{42,43} β -catenin translocates to the cellular nucleus of hepatocytes, where it induces the transcription of Wnt target genes, responsible for cell proliferation.⁴⁴ Simultaneously, the Notch pathway is stimulated, leading to the nuclear translocation of NICD (Notch intracellular domain).⁴⁵ The Wnt/ β -catenin and Notch pathways are implicated in the epithelial-to-mesenchymal transition (EMT),⁴⁶ a process required for liver regeneration.

The increased concentration of LPS activates Kupffer cells through Toll-like receptor 4 (TLR4), C3a and C35 receptors. Similarly to what happens in response to IRI, this leads to NF- κ B nuclear translocation with subsequent secretion of tumour necrosis factor- α (TNF- α) and interleukin (IL)-6.^{47,48} TNF- α further sustains the activation of the NF- κ B pathway and the production of IL-6. IL-6 binds to the IL-6 receptor on hepatocytes and induces STAT3 (signal transducer and activator of transcription-3), which is responsible for hepatocyte proliferation through cyclin induction.^{47,49}

Proliferation

The proliferation phase is characterized by cell proliferation in the first 72 h, followed by angiogenesis. Several growth factors are involved in the proliferation phase, including HGF,⁵⁰ epidermal growth factor (EGF),^{51,52} and vascular endothelial growth factor (VEGF).⁵³ HGF and EGF, together with other signalling molecules, activate the IL-6/JAK/STAT3, Ras/MAPK, and PI3-K/PDK1/Akt pathways, which contribute to cell proliferation and cell growth.⁵⁴ VEGF is involved in angiogenesis and reconstitution of the sinusoids after cell proliferation.⁵⁵

Termination

Regeneration is terminated when the liver reaches its pre-hepatectomy size, or a 2.5% liver mass to total body mass ratio is restored.⁵⁶ Any further proliferation is resolved by apoptosis. The main known factors responsible for termination are TGF- β ,^{57–59} activin^{60,61} and the Hippo/Yap/Taz pathway,⁶² which act in a similar manner to limit hepatocyte proliferation.⁶³

Ischaemia-reperfusion injury

The full spectrum of IRI occurs whenever blood flow is suddenly restored after a period of ischaemia. During ischaemia, a lack of oxygen causes the mitochondrial electron transport chain to stop producing ATP, causing ATP depletion, acidosis and cell oedema.⁶⁴ The components of the electron transport chain get saturated with electrons, as oxygen is not available for oxidative phosphorylation, and tricarboxylic acid (TCA) intermediates, including NADH and succinate, accumulate. This impaired mitochondrial respiration causes the well-described calcium release into the cytosol,⁶⁵ which triggers the opening of the mitochondrial permeability transition pore and initiates cell death. When the liver circulation is re-established, the sudden availability of high succinate levels in hypoxic tissue leads to a massive production of reactive oxygen species (ROS) at complex I, which is mainly based on complex II dysfunction. Apoptosis/necrosis, endothelial dysfunction, and inflammation are the direct consequence (Fig. 1).⁶⁶ Excessive oxidative stress therefore causes nuclear DNA damage, protein oxidation, and further mitochondrial permeability transition pore opening, leading to cell death⁶⁷ and the subsequent release of damage-associated molecular patterns (DAMPs). DAMPs further push the establishment of an inflammatory microenvironment via activation of the inflammasome,⁶⁶ the release of inflammatory cytokines and complement proteins, and immune cell activation.^{68,69} Both, the innate and the adaptive immune response play a major role in IRI pathways and link liver quality to acute liver rejection.^{70,71}

Hepatic ischaemia-reperfusion injury - mechanism

In liver transplantation, IRI is an inevitable consequence of conventional organ procurement. For deceased donors, this process occurs during donor evaluation in the intensive care unit, where loss of brain function is often accompanied by some haemodynamic instability with associated periods of hypotension and hypoxia. The procurement itself involves a period of CIT for donation after brain death (DBD) or a period of warm ischaemia time (WIT) followed by CIT in DCD. While LDLT is commonly regarded as an almost ischaemia-free procedure, a certain IRI can also be observed in this setting due to parenchymal damage during liver transection, elective use of the Pringle manoeuvre, liver manipulation, and CIT during graft

back-table preparation. The graft experiences further warm ischaemia during implantation, followed by reperfusion.

IRI occurs not only during transplantation, but during liver resection as well. As we have described for LDLT, liver surgery involves hypoxic and ischaemic damage to the liver parenchyma during vascular occlusion, manipulation, and transection.

Hepatic IRI can be divided into an acute phase, lasting approximately 3 h after reperfusion, and a late phase lasting up to 48 h after reperfusion. The acute phase is characterized by mitochondrial dysfunction causing oxidative stress in hepatocytes, Kupffer cells, and sinusoidal epithelial cells. Oxidative stress induces the release of HMGB1 (high-mobility group box 1),⁷² a DAMP that mediates Kupffer cell activation through a TLR4-mediated pathway. Activated Kupffer cells produce more ROS, which induce the degradation of I κ B, the molecule that inhibits the translocation of NF- κ B from the cytosol to the cellular nucleus.^{73,74} NF- κ B is an important mediator of hepatic IRI.^{75–77} Once freed, NF- κ B enters the nucleus and upregulates the synthesis of mediators of the pro-inflammatory cytokine cascade, including IL-1 β , IL-6, and TNF- α .^{74,78} Pro-inflammatory cytokines act on adhesion molecules and complement proteins to attract neutrophils and CD4+ T cells into the liver, which are the main mediators of the late phase of IRI. Neutrophils increase liver damage through secretion of ROS, MMPs, and proteases^{79–81} causing hepatocyte necrosis. This liver damage can eventually evolve into chronic inflammation, causing long-term consequences such as liver fibrosis and, as we will further explore in the following sections, cancer recurrence.

Hepatic ischaemia-reperfusion injury – clinical implications

IRI is not merely a time-dependent process, but its severity is also related to the characteristics of the liver graft and the recipient. Grafts from extended criteria donors are at an increased risk of more severe IRI after liver transplantation.⁸² Steatotic liver grafts exhibit comparatively impaired regenerative responses and reduced tolerance to IRI,⁸³ which translates into higher rates of primary non function and early allograft dysfunction.^{84,85} DCD liver grafts have a higher rate of microthrombi formation⁸⁶ and cell damage related to WIT.⁸⁷ The age of the donor is also a risk factor for more severe IRI, with age >70 years being associated with smaller volumes, reduced overall flow,⁸⁸ and less mitochondria.⁸⁹ Small for size grafts^{90,91} are also a risk factor, a relevant consideration in LDLT. Intuitively, the combination of any of these risk factors further exacerbates IRI severity, as demonstrated by reports that prolonged CIT is especially deleterious in steatotic livers.⁹²

Recipient risk factors for more severe IRI are mainly related to cirrhosis⁹³ and subsequent altered coagulation profiles,⁹⁴ which

Box 1. Critical commentary on cancer recurrence and intraoperative factors.

Liver surgery requires a strong surgeon-anaesthesiologist relationship, more so than other anatomical districts. Adequate perioperative management is paramount to maintain fluid balance, decrease surgical stress and accelerate postoperative recovery, as well as provide an optimal oncological outcome. Recent evidence suggests that the impact of the surgical act on prognosis is not a mere matter of surgical margins, but that every step of the surgical procedure can influence tumour recurrence. All efforts should thus be taken to decrease the intraoperative spread of cancer cells and mitigate the post-surgical pro-inflammatory state.

lead to a pro-thrombotic state with low platelets, altered platelet aggregation and altered production of coagulation factors.⁹⁵

Similar considerations can be applied to the quality of the liver parenchyma during liver resection, where patients who are older and/or have a higher degree of steatosis or cirrhosis have less tolerance for vascular occlusion and IRI. IRI is also associated with impaired liver regeneration through mechanisms that will be explored in the following section.

Tumour recurrence after liver resection and transplantation

Tumour recurrence after liver surgery is likely linked to the mobilization of circulating tumour cells (CTCs), activation of dormant micrometastases, or a combination of the two. Both

resection and transplantation cause major alterations in the local microenvironment and at the systemic level, with activation of pathways that favour the engraftment of residual tumour cells.

The link between cancer recurrence and perioperative acute liver injury is now well accepted. The main processes that tie these two events are mechanical handling, response to IRI, and regeneration (Fig. 2). Mechanical handling and squeezing of the liver during surgery may lead to the release of cancer cells into the systemic circulation. IRI, causing mitochondrial dysfunction, can activate many processes that characterize “oncogenic mitochondria”, together with an inflammatory microenvironment that promotes tumour cell mobilization and engraftment. Finally, in liver regeneration, the drivers of hepatocyte differentiation

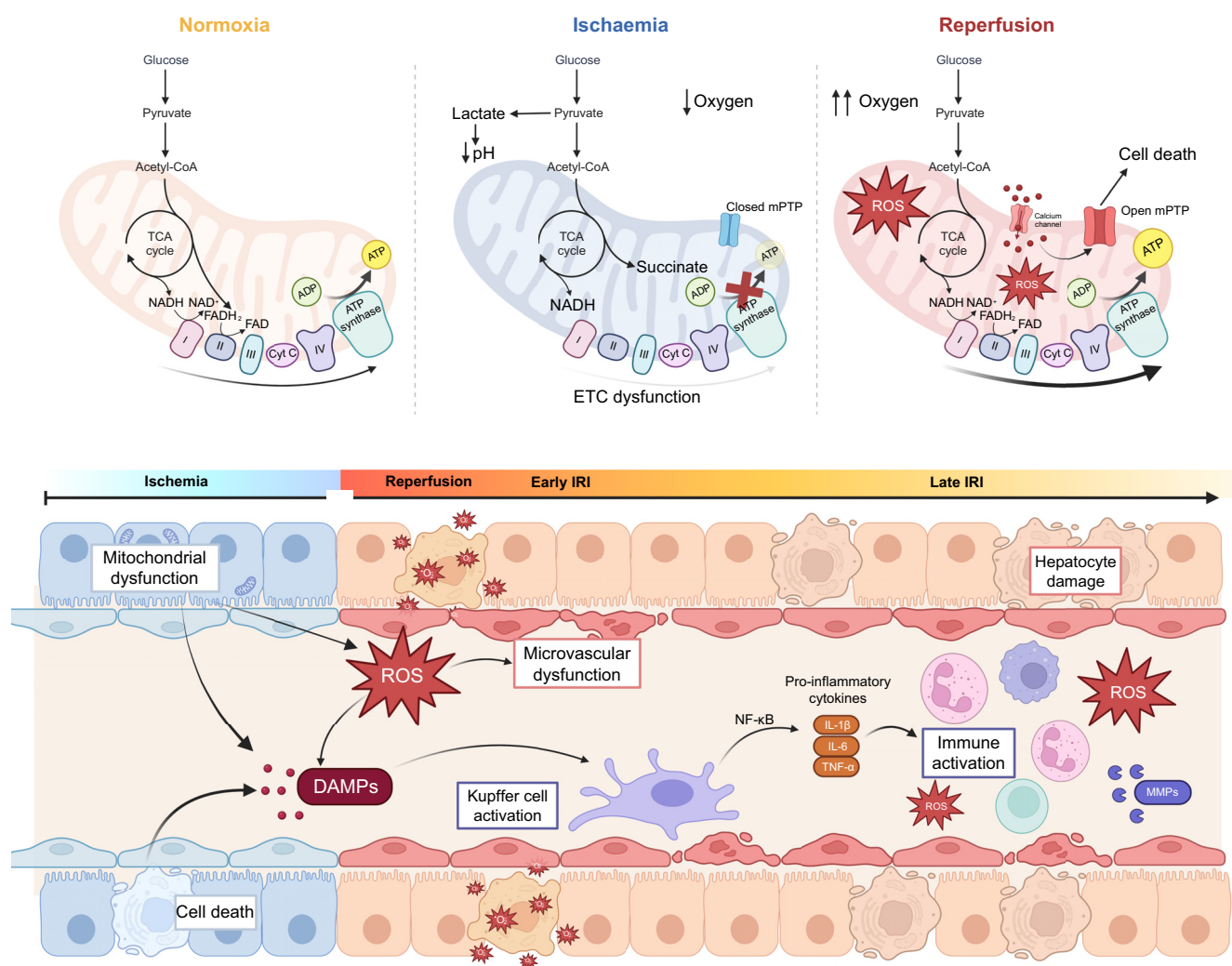


Fig. 1. Ischaemia-reperfusion injury. (A) Mitochondrial metabolism during normoxia. During normoxia, the mitochondria exert their physiological functions, in which byproducts of the TCA cycle enter the ETC to be used as substrates for oxidative phosphorylation and the conversion of oxygen to ATP. (B) Mitochondrial metabolism during hypoxia. During hypoxia, the TCA continues operating as usual, while the ETC malfunctions due to the lack of oxygen. NADH and succinate accumulate, an important concept for reperfusion. (C) Mitochondrial metabolism during reperfusion. At reperfusion, the influx of oxygen overwhelms the capacity of the dysfunctional ETC to metabolize it, leading to production of ROS and opening of the mPTP. The open mPTP stimulates cell death. (D) Main mechanisms involved in IRI. Ischaemia is characterized by mitochondrial dysfunction: the cells are not able to function as they do in normoxic conditions, and cells begin to die. After reperfusion, the release of ROS leads to microvascular dysfunction, release of damage-associated molecular patterns, and Kupffer cell activation. In the late part of IRI, the immune system is activated with influx of neutrophils, macrophages and T cells, further exacerbating ROS and hepatocyte damage. ETC, electron transport chain; IRI, ischaemia-reperfusion injury; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species; TCA, tricarboxylic acid.

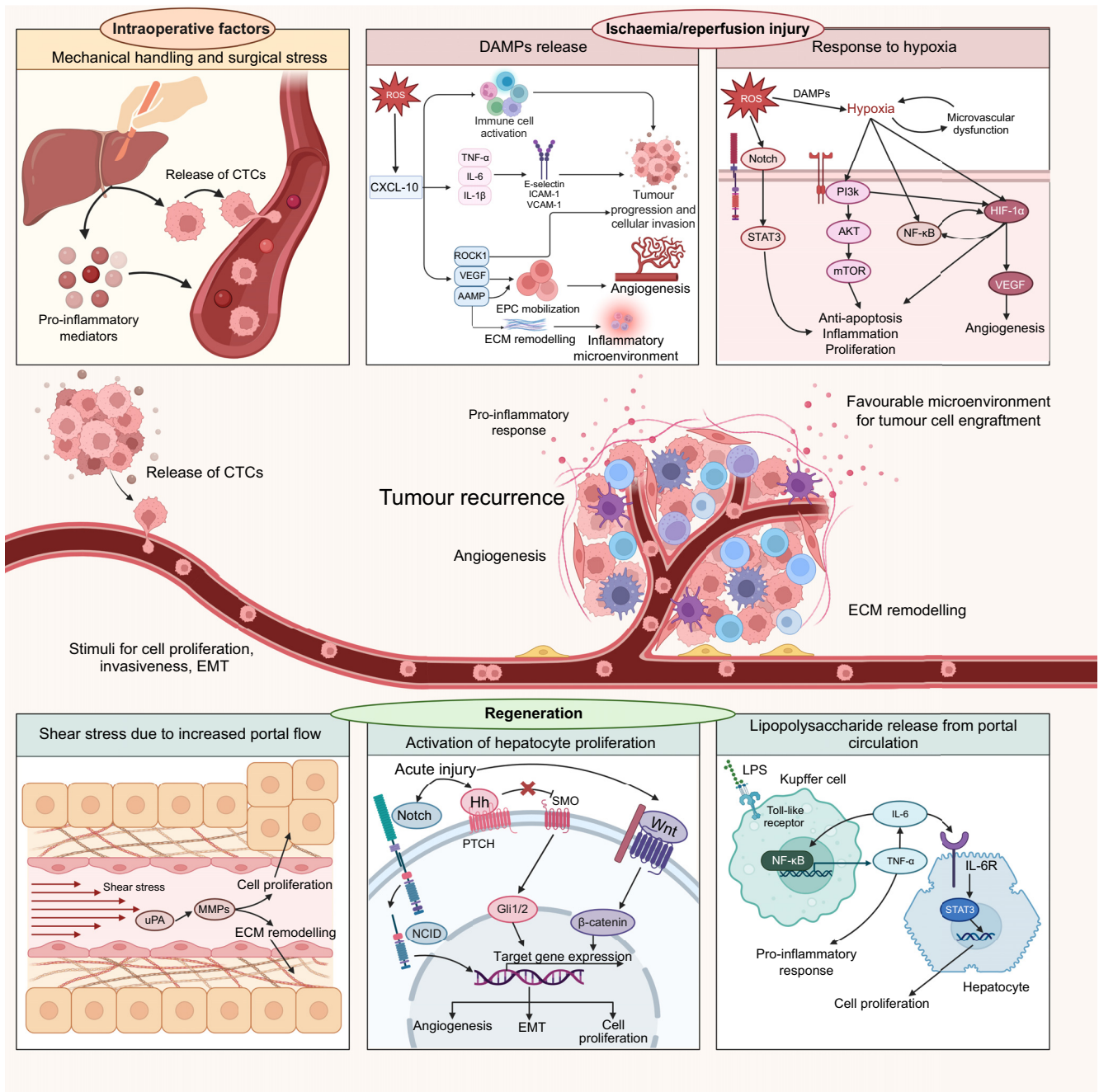


Fig. 2. The link between intraoperative injury, ischaemia-reperfusion injury, and regeneration. The contribution of perioperative liver injury to tumour recurrence involves the release of tumour cells into an environment that favours tumour engraftment, similarly to the seed and soil theory. Mechanical handling of the liver promotes the release of CTCs (the “seed”) and pro-inflammatory mediators into the bloodstream. Ischaemia-reperfusion injury causes necrosis and oxidative stress, leading to the release of DAMPs, cytokines, adhesion molecules, and exacerbation of hypoxic conditions. The downstream pathways activated as a consequence are responsible for tumour progression, angiogenesis, and creation of a pro-inflammatory microenvironment that favours tumour engraftment (the “soil”). Finally, regenerative pathways are triggered, stimulating cell proliferation, epithelial-to-mesenchymal transition, remodelling of the ECM, and angiogenesis, i.e. mechanisms linked to tumour growth and invasiveness. CTCs, circulating tumour cells; DAMPs, damage-associated molecular patterns; ECM, extracellular matrix; EPCs, endothelial progenitor cells; ROS, reactive oxygen species.

and hyperplasia may also become drivers of cancer recurrence, as well as promoting factors for the reactivation of dormant stem cells. Each method of transplantation and resection is related to a different degree of any of these three processes (Table 1).

Cancer recurrence and intraoperative factors (Box 1)

The surgical technique has an impact on the pro-inflammatory microenvironment that develops in the perioperative period. The intraoperative handling and “squeezing of the liver” can

Table 1. Level of perioperative liver injury seen with different resection and liver transplant techniques.

	Mechanical handling	Ischemia/reperfusion injury	Regeneration
Liver transplantation			
DBD - whole	++	+ to +++ [†]	+
DBD - split	++	++ to +++	++
DCD - whole	++	++ to +++	+
DCD - split	++	+++	(+)
LDLT	++	+	++
Auxiliary	++	++	++
Liver resection			
Minor liver resection	+	+	+
Major liver resection (one stage procedure)	+++	++	+++
Two-stage hepatectomy* ± PVE	+++	++	++
ALPPS	+++	++	+++
Combine techniques			
RAPID	+++	++	++ to +++

+ = minor; ++ = moderate; +++ = major.

ALPPS, associated liver partition and portal vein ligation for staged-hepatectomy; DBD, donation after brain death; DCD, donation after cardiac death; LDLT, living donor liver transplantation; LT, liver transplantation; PVE, portal vein embolization; RAPID, resection and partial liver segment 2–3 transplantation with delayed total hepatectomy (residual hepatectomy when transplanted segment 2–3 have grown sufficiently = the second stage); TSH, two-stage hepatectomy.

* Traditional approach: portal vein ligation and partial liver resection (*i.e.*, wedge resection, segmentectomy or left lateral sectionectomy resection).

[†] In whole DBD grafts, the amount of IRI greatly depends on donor-associated risk factors. High-risk donors (*i.e.*, donor on extracorporeal life support, steatotic liver, need for high pressor support, repeated episodes of hypotension before or during procurement) will trigger more IRI compared to low-risk donors (*i.e.*, young and healthy donors, traumatic cause of death, clinically and medically stable before procurement).

facilitate the mobilization of tumour cells, turning them into CTCs, as well as increasing the release of catecholamines, prostaglandins, and other inflammatory mediators.⁹⁶ Catecholamines and prostaglandins have been linked to metastasis development. Norepinephrine and pro-inflammatory cytokines (IL-6, TNF- α) can stimulate VEGF-mediated angiogenesis.⁹⁷ The increase in pro-inflammatory mediators and growth factors triggers cellular proliferation in an environment which favours motility and invasiveness. In a study comparing anaesthesia alone to laparotomy, appendectomy, and liver resection in a mouse model of colorectal cancer (CRC), concomitant lung metastases increased proportionally to the degree of surgical stress.⁹⁸ In a retrospective analysis of 106 patients undergoing liver transplantation for HCC,⁹⁹ 59 patients were treated with PGE-1 (prostaglandin E1) to diminish surgical stress and IRI; 3- and 5-year recurrence-free survival was significantly higher in the PGE-1-treated group vs. the untreated group (87.9% and 85.7%, vs. 65.3% and 63.1%, respectively, $p = 0.003$). Further prospective clinical studies are required to establish this beneficial effect.

Techniques to minimize liver handling during resection have been developed, including the anterior approach to major hepatectomy. Unlike the conventional technique, which requires complete mobilization of the right hepatic lobe for a right hepatectomy, the anterior approach involves early inflow control, the completion of parenchymal transection, and outflow control before liver lobe mobilization.¹⁰⁰ The anterior approach reduced liver handling-induced mobilization of tumour cells and has been associated with better survival outcomes, especially disease-free survival, after resection.^{100,101} This relates to the concept that surgeon's experience may also be associated with cancer recurrence.¹⁰² A technically "cleaner" operation performed by an experienced surgeon may cause less tissue trauma, oedema, blood loss, release of inflammatory mediators and CTCs, all factors that play a role in postoperative cancer recurrence.¹⁰³

The surgical act is not the only intraoperative event with the potential to promote cancer recurrence. Other factors, such as anaesthetic drugs, hypothermia, and transfusion of blood products also increase the inflammatory response.¹⁰³ They increase secretion of catecholamines and cytokines, promote platelet activation, and contribute to the immunosuppressed status that

is expected after surgery. These changes increase the chances that released CTCs will be able to escape immunological recognition and encounter a favourable microenvironment for homing and metastasis.

Cancer recurrence and regeneration - mechanism

The link between regeneration and recurrence is an intuitive one: regeneration involves cellular proliferation in an inflammatory microenvironment, the ideal conditions for tumour proliferation, and many pathways involved in liver regeneration play a role in cancer development, progression, and recurrence.

The interplay between regeneration and cancer recurrence occurs through regulation of cellular proliferation, EMT, angiogenesis, breakdown and remodelling of the extracellular matrix (ECM), and activation of CTCs (Fig. 3).

The Wnt/ β -catenin, Notch, and Hedgehog pathways,^{46,104} as well as the LPS-mediated release of pro-inflammatory cytokines (IL-6, IL-1 β , TNF- α) are responsible for cellular proliferation and EMT.¹⁰⁵ Notch signalling has an established role in HCC¹⁰⁶ and metastatic CRC development;¹⁰⁴ it promotes secretion of growth cytokines responsible for cell proliferation, EMT, and angiogenesis. Hedgehog is another regulator of cell proliferation and EMT, which is highly expressed in HCC.¹⁰⁷ Finally, β -catenin is upregulated in up to 35% of HCCs.⁴⁶ Mutations in APC (adenomatous polyposis coli), which occur in the majority of cases of CRC, cause irreversible activation of this pathway.^{108,109} β -catenin also regulates the Hippo/Yap/Taz pathway through Yap activation.¹¹⁰ The oncogenic role of Yap has been linked to HCC,¹¹¹ CRC,¹¹² and cholangiocarcinoma.¹¹³

Hippo/Yap/Taz is not the only pathway regulating termination that is involved in carcinogenesis. TGF- β can play both a pro- and an anti-tumorigenic role depending on the timing and the characteristics of the microenvironment. It exhibits anti-carcinogenic effects in the early stages of tumour development. This is followed by a shift, the mechanism of which is poorly understood, in which TGF- β becomes a key player in carcinogenesis and metastatic development.^{114,115} This phenomenon is well studied in metastatic CRC: the overexpression of TGF- β causes EMT, acquisition of stem cell-like features in cancer cells, and stromal transformation into a pro-carcinogenic

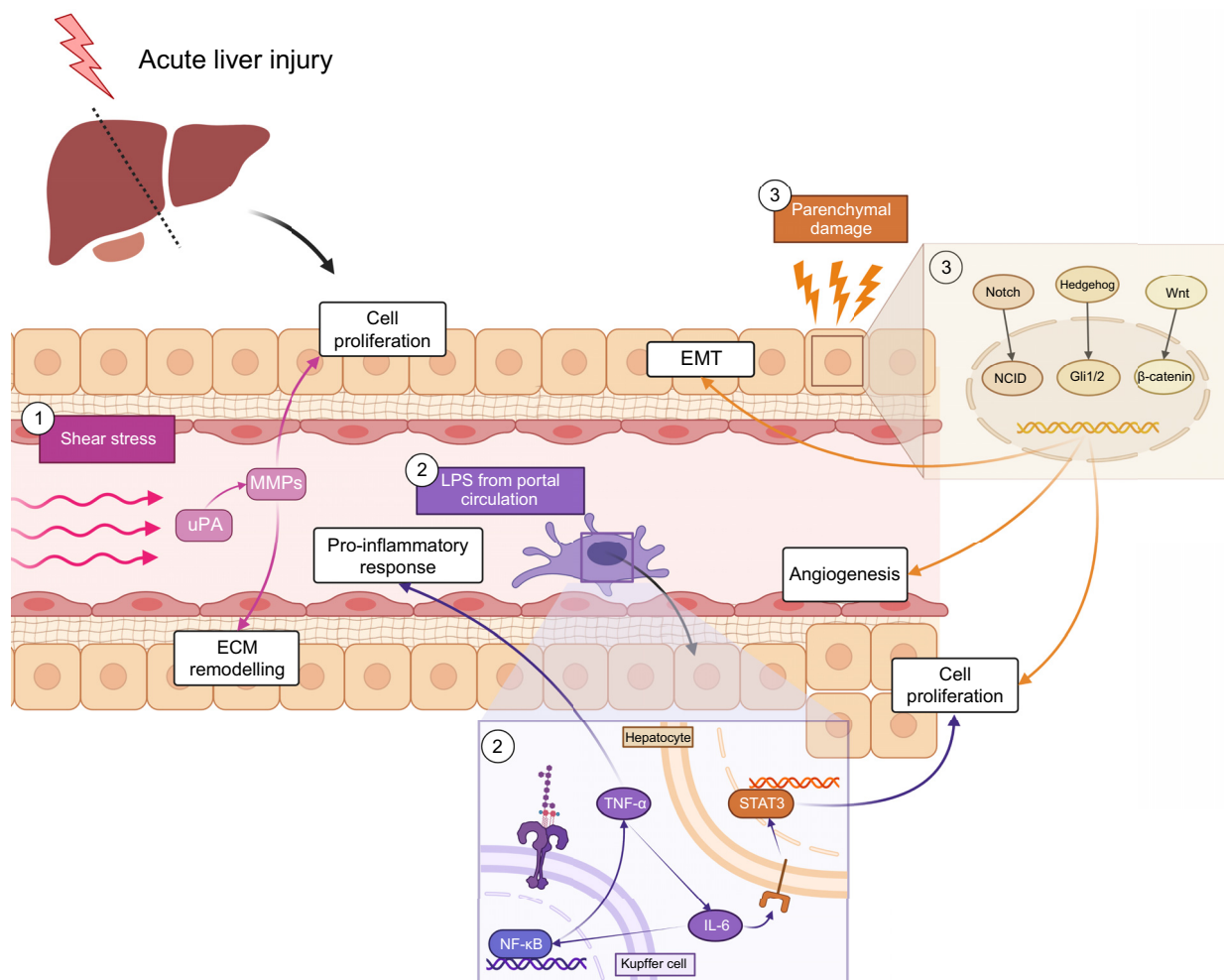


Fig. 3. The link between liver regeneration and tumour recurrence. Parenchymal loss and transplantation of a small-for-size graft are major triggers of liver regeneration. Liver regeneration is activated by the increase in portal flow per unit, which causes shear stress and an increase in the concentration of LPS. Shear stress-activated uroplasinogen and matrix metalloproteinases, responsible for cell proliferation through secretion of growth factors and remodelling of the ECM. The increase in LPS stimulates the nuclear localization of NF-κB, which can then exert its effect on the transcription of target genes. These genes modulate the secretion of pro-inflammatory cytokines such as TNF-α and IL-6, which is also responsible for STAT3 activation in hepatocytes, leading to their proliferation. Injury also affects hepatocytes, activating three pathways (Notch, Hedgehog, Wnt/β-catenin) which favour EMT, angiogenesis and cell proliferation. ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; LPS, lipopolysaccharide.

microenvironment, driving metastatic development.^{116,117} Metastatic and invasive CRC cancer cells have higher levels of TGF-β compared to cancer cells in the primary tumour,¹¹⁸ and higher postoperative levels of TGF-β have been shown to correlate with higher risk of recurrence after curative resection.¹¹⁷

EMT and subsequent MET (mesenchymal-to-epithelial transition) after cancer homing are pivotal for metastatic development. CTCs can be both epithelial or mesenchymal cells, or exhibit features of both,^{119,120} as influenced by the Wnt/β-catenin, TGF-β, and Hedgehog pathways. Growth factors, such as HGF and EGF, also play a role in EMT.¹²¹

HGF and EGF are growth factors that sustain the proliferative phase of regeneration. EGF has been linked to proliferation of CRC metastases, and its receptor, EGFR, is a known therapeutic target. Cetuximab and panitumumab are anti-EGFR monoclonal antibodies used in metastatic CRC either in combination with chemotherapy or as monotherapy.^{122,123} HGF is a major driver of hepatocyte proliferation during liver regeneration. Postoperative HGF elevation is directly correlated with growth of the liver remnant after hepatectomy, ALPPS, and LDLT.^{124–126} Higher

postoperative plasmatic concentrations of HGF are also associated with higher recurrence of metastatic CRC,¹²⁷ and over-expression of the HGF receptor c-Met has been linked to worsened prognosis and more aggressive features.^{128,129} HGF is also responsible for activating the STAT3 pathway,¹³⁰ whose activation is increased post-hepatectomy and promotes cancer recurrence, as well as enhancing the secretion and activity of MMPs.^{131,132}

MMPs, together with urokinase plasminogen activator and other proteases, are responsible for ECM remodelling,¹³³ a crucial process in liver regeneration, that promotes both migration and adhesion of cells during cellular proliferation. Likewise, ECM breakdown and remodelling may promote the shedding of tumour cells into the circulation, with subsequent homing either in the liver remnant or in distant organs. Of note, MMPs are also important mediators of tissue injury after IRI¹³³ by promoting apoptosis, endothelial dysfunction, leukocyte migration into the liver, and sustaining the secretion of pro-inflammatory molecules. MMPs also play a role in angiogenesis, together with Notch^{134,135} and VEGF.¹³⁶ VEGF receptors are the targets of

several anti-angiogenic therapeutics used in advanced HCC,¹³⁶ including the tyrosine kinase inhibitors sorafenib, regorafenib, sunitinib and lenvatinib, and the monoclonal antibody ramucirumab. Cabozantinib, a second-line tyrosine kinase inhibitor targets both VEGFR2 and c-Met (the HGF receptor).¹³⁷ Anti-angiogenic therapies are also used in both HCC¹³⁸ and metastatic CRC,¹³⁹ with the most common and well-studied agent being the monoclonal antibody bevacizumab.

In summary, liver regeneration requires extensive cellular activation and proliferation in a microenvironment that favours cancer development and metastases thanks to ECM remodelling, angiogenesis, and inflammation.

Cancer recurrence and regeneration – clinical implications (Box 2)

Animal models have clearly demonstrated the relationship between post-hepatectomy regeneration and tumour growth. In mouse models of CRC with extrahepatic metastases, the post-hepatectomy growth of extrahepatic lesions exceeded that of the liver remnant.^{140,141} This effect was observed after major resections, which required regeneration to maintain liver function, but not after minor resections, when the need for subsequent regeneration was limited.^{141,142}

The accelerated growth of cancer tissue compared to the liver has implications for all procedures that require liver hypertrophy with the bulk of the tumour still in place, such as TSH, PVE, ALPPS and auxiliary liver transplantation. These techniques operate on the fine balance between adequate liver hypertrophy and risk of dropout due to tumour progression. The timeframe needed for liver remnant hypertrophy is different with each technique, with TSH requiring up to 3 months, PVE between 4 and 6 weeks, and ALPPS 2 to 3 weeks. This difference in timing is reflected by dropout rates. The dropout rate due to tumour progression is between 28–38%^{143,144} after the first stage of TSH and around 30% after PVE,¹⁴⁵ while it is much lower after the first stage of ALPPS.¹⁴⁶ The latter, in particular, is mostly driven by postoperative complications rather than tumour progression.^{147,148}

Whether this interim tumour growth has long-term prognostic implications once the second stage is performed is still to be determined. Growth of liver metastases and even development of new lesions after PVE have been documented both in animal models¹⁴⁹ and humans.¹⁵⁰ Despite inconsistent reports on the long-term outcomes of PVE, a recent meta-analysis has shown that, once definitive resection is performed, PVE has no detrimental effect on intrahepatic recurrence or overall survival.¹⁵¹

Similar questions can be posed after partial and auxiliary liver transplantation for oncological indications. Patients with cancer are considered ideal candidates for these grafts, as they usually have a preserved liver function and can support the necessary regeneration process better than patients undergoing transplantation for end-stage liver disease. LDLT is often performed with small-for-size grafts, and always requires at least some level of liver regeneration. The evidence on whether HCC recurrence is higher after LDLT compared to deceased donor transplantation is inconclusive, with conflicting reports.^{152,153} This likely reflects the multifactorial nature of cancer recurrence as well as the limitations of comparing two interventions that are not necessarily offered to the same patient populations.¹⁵⁴ The contribution of small-for-size liver grafts to cancer recurrence is controversial, with some retrospective analyses reporting higher

recurrence,¹⁵⁵ while others report no difference.^{156,157} The experience on auxiliary transplantation is still scarce, but the risk of tumour progression between the two stages will be a matter of interest as this technique gains traction.

Box 2. Critical commentary on tumour recurrence and liver regeneration.

Its capacity for regeneration is one of the most extraordinary features of the liver and a cornerstone of liver surgery. The dramatic influx of factors promoting differentiation and angiogenesis, paired with surgery-induced and drug-induced immunosuppression, may however contribute to the growth and metastasis of residual cancer cells. As the boundaries of liver surgery and transplantation are pushed further and further, the need to increase our understanding of the regeneration process and its regulatory mechanisms becomes more pressing. On one hand, we should gather more insights on the factors that drive or impair regeneration, hopefully on a deeper level than the state of the parenchyma or the age and metabolic reserve of the patient. On the other, we should find ways to selectively promote parenchymal growth while inhibiting or restricting any concomitant tumour growth.

Underlying mechanisms linking cancer recurrence and ischaemia-reperfusion injury

Tumorigenesis and IRI share several downstream pathways and a similar microenvironment, thus it is unsurprising that IRI increases the risk of cancer recurrence. The degree of IRI is clearly linked to the duration of hypoxia and vascular occlusion, but there are other exacerbating factors related to the quality of the liver parenchyma, e.g. age of the patient or donor and the degree of steatosis.²⁰ Higher IRI levels are associated with more tumour recurrence and a more invasive phenotype of cancer cells.

The main drivers of IRI are cell death and mitochondrial dysfunction, with subsequent production of ROS and oxidative stress. As shown in Fig. 4, oxidative stress leads to release of DAMPs, secretion of inflammatory cytokines, and subsequent microvascular dysfunction.

DAMPs activate C-X-C motif chemokine ligand 10 (CXCL10) through a TLR4-mediated mechanism.¹⁵⁸ CXCL10 is a major driver of the pro-inflammatory response to IRI with implications for cancer recurrence. It attracts macrophages, monocytes, regulatory B cells and regulatory T cells to the liver, which have been shown to promote tumour progression and cancer cell invasion.^{159–161} CXCL10 regulates the secretion of inflammatory cytokines, including TNF- α , IL-6 and IL-1 β .¹⁵⁸ These inflammatory cytokines increase the expression of adhesion molecules (e.g. E-selectin, ICAM-1, VCAM-1), which act as mediators of tumour growth and engraftment. CXCL10 also has a direct effect on the aggressive phenotype of tumour cells, increasing cell motility and invasiveness⁹⁰ by upregulating ROCK1 (Rho-associated protein kinase-1), VEGF and MMP. Overexpression of ROCK, which regulates cellular motility, proliferation, and apoptosis, leads to infiltrative tumour growth and metastasis.¹⁶² VEGF and MMP play a role in recruiting endothelial progenitor cells^{163,164} from the bone marrow, stimulating neoangiogenesis and creating favourable conditions for recurrence. Furthermore, the expression of MMPs, particularly MMP-9, is pivotal for ECM degradation and remodelling,¹⁶⁵ a key step in the recruitment and transmigration of neutrophils and other immune cells into the liver after IRI. Neutrophils and immune cells further aggravate liver damage and ROS production, contributing to a

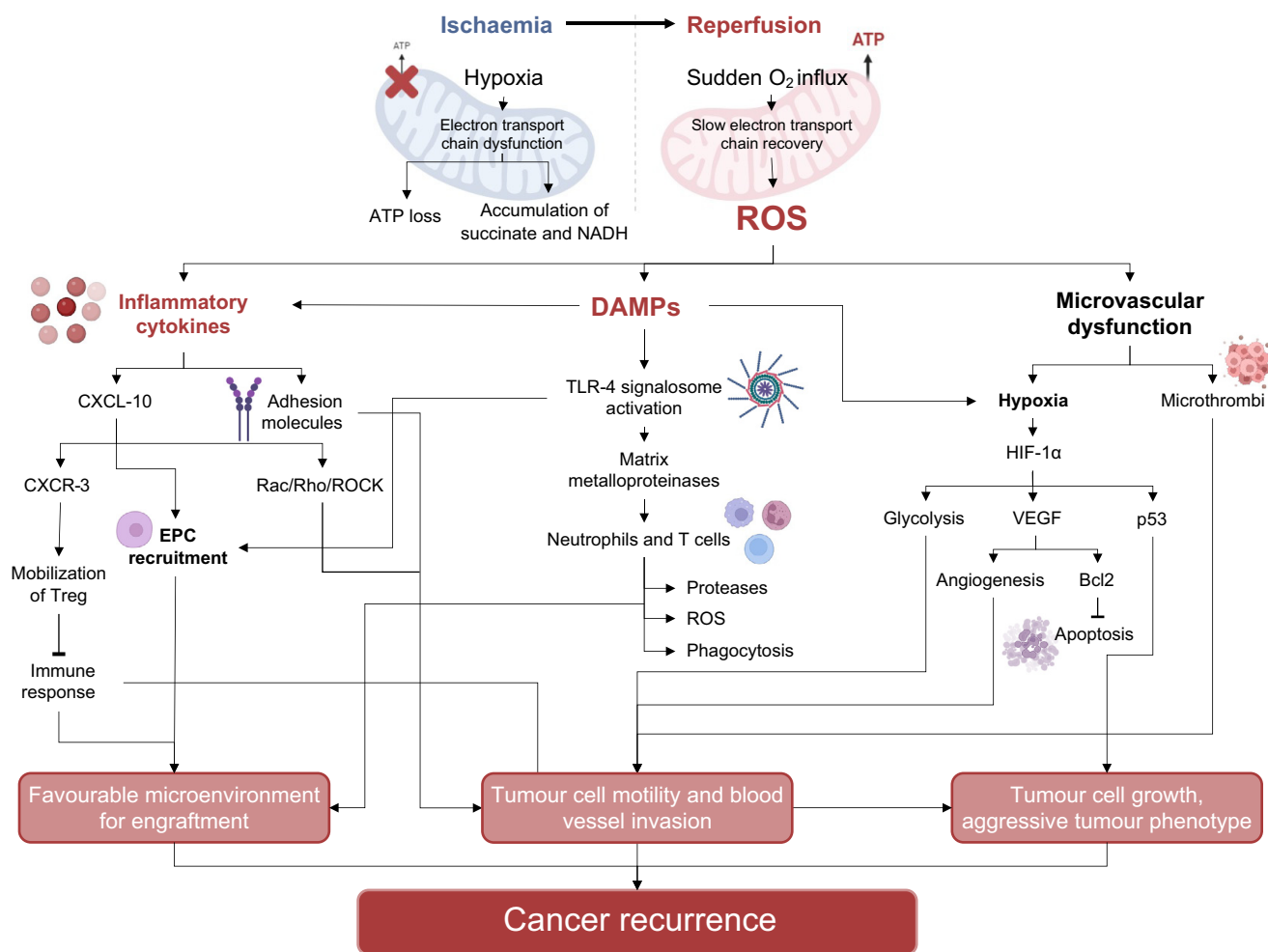


Fig. 4. The link between ischaemia-reperfusion injury and tumour recurrence. During ischaemia, dysfunction in the ETC caused by lack of oxygen leads to ATP loss and accumulation of TCA cycle products, such as succinate and NADH. Subsequent reperfusion involves the sudden influx of high quantities of oxygen; the ETC cannot recover fast enough to deal with this oxygen influx, causing the production of a massive amount of ROS. Oxidative stress triggers the release of DAMPs and inflammatory cytokines, and microvascular dysfunction. DAMPs and inflammatory cytokines are responsible for recruitment of endothelial progenitor cells, pathways stimulating cell differentiation, and matrix remodelling through metalloproteinases. They also attract neutrophils and T cells, further stimulating ROS and proteases and contributing to the development of a favourable microenvironment for engraftment. Microvascular dysfunction, ROS and DAMPs further exacerbate hypoxia, leading to the activation of HIF-1 α , which stimulates angiogenesis, anti-apoptotic signals, cell growth and glycolysis. As a consequence, tumour cells receive stimuli to proliferate and invade the local and systemic circulation with an aggressive phenotype. DAMPs, damage-associated molecular patterns; EPCs, endothelial progenitor cells; ETC, electron transport chain; ROS, reactive oxygen species.

favourable microenvironment for cancer proliferation and recurrence. It must be noted, however, that recent evidence shows that the role of neutrophils in liver transplant IRI is not merely pro-inflammatory.⁸⁰ Specific subpopulations of neutrophils have been demonstrated to promote clearance of inflammation, revascularization and tissue repair.¹⁶⁶

The hypoxic conditions during IRI cause endothelial cell swelling and unbalanced vasoconstriction, leading to microvascular dysfunction. Microvascular dysfunction, together with DAMP release, exacerbates tissue hypoxia, leading to hypoxia-inducible factor-1 α (HIF-1 α) activation. After nuclear translocation, HIF-1 α upregulates the expression of genes related to angiogenesis, glycolytic switch, cell proliferation, and apoptosis,^{167,168} which can stimulate tumour growth and metastatic potential. The role of HIF-1 α in the regulation of hypoxia-induced apoptosis and cell death is controversial. During hypoxia, HIF-1 α promotes hypoxia-induced apoptosis via caspase activation and mitochondrial cytochrome c release;¹⁶⁹ this

happens through different pathways, including p53 stabilization and induction of proteins, such as BNIP3 (Bcl-2 interacting protein 3), that downregulate the anti-apoptotic Bcl-2 (B-cell lymphoma 2). However, HIF-1 α may also have anti-apoptotic effects, e.g. by promoting the transcription of genes that inhibit apoptosis, such as IAP2 (inhibitor of apoptosis protein 2). The target genes upregulated by HIF-1 α in any given cell seem to depend on cell type and on the microenvironment. HIF-1 α also plays a role in the regulation of mitochondrial metabolism. At low oxygen levels, HIF-1 α decreases the activity of the TCA cycle,¹⁷⁰ thus decreasing oxygen consumption, and stimulates glycolysis and autophagy, mechanisms required for cell survival in hypoxic conditions and often present in cancer cells. The presence of HIF-1 α in several cancers has been linked to clonal selection of hypoxia-resistant cancer cells,¹⁷¹ and its expression is associated with resistance to treatment. Increased levels of HIF-1 α have been linked to poorer prognosis in HCC¹⁷² and post-surgical recurrence in CRLMs.¹⁷³ Indeed, hypoxia-induced HIF-1 α

promotes EMT in CRC cells.¹⁷⁴ In addition to HIF-1 α expression, hypoxia may also prevent apoptosis through induction of the PI3K/Akt signalling pathway, which regulates cell survival and proliferation,¹⁷⁵ as well as NF- κ B¹⁷⁶ and STAT3.¹⁷⁷ Notch, whose instigation is mediated by ROS, also stimulates STAT3 activation in a mechanism common to both IRI and regeneration, promoting hepatocyte proliferation.¹⁷⁸

Cancer recurrence and ischaemia-reperfusion injury – clinical and preclinical evidence and implications (Box 3)

Evidence that IRI stimulates tumour growth and recurrence comes from animal models, as well as from post-hepatectomy and post-transplantation observations in humans.

Preclinical evidence

In animal models, IRI exerts an effect on both primary and secondary tumours. In a study by Orci *et al.*,¹⁷⁹ the increase in LPS as a consequence of reperfusion after vascular clamping was responsible for higher HCC burden through a TLR4-mediated pathway, in a mechanism shared by both IRI and liver regeneration, and activated during both liver resection with the Pringle manoeuvre and liver transplantation. Oldani *et al.*¹⁸⁰ showed that HCC growth was higher after transplantation with an ischaemic graft, while this effect was reversed if the graft was reperfused prior to implantation. The duration of ischaemia correlated with HCC growth. In a study by the same group,¹⁸¹ HCC recurrence was worse after IRI in mice with severe steatosis compared to controls, and expression of inflammatory genes encoding for IL-6, TNF- α , HIF-1 α and E-selectin was upregulated. Similarly, Yang *et al.*¹⁸² found that IRI induces HCC recurrence in fatty livers by inducing EMT and MMP activation through a PI3K/AKT/NF- κ B-mediated pathway. These findings highlight the pivotal role of parenchymal quality on IRI tolerance.

Similar evidence has been found for CRLMs. van der Bilt *et al.*¹⁸³ reported the accelerated growth of CRLMs after vascular clamping, which was associated with areas of hepatic necrosis in an inflammatory microenvironment. This effect, observed with prolonged vascular clamping, was not present with intermittent vascular clamping. The same group¹⁸⁴ found that IRI-accelerated CRLM growth was exacerbated by increasing ischaemia time, and further increased with age and steatosis. Different groups have reported that targeting byproducts of the cascades initiated by IRI, such as MMP-9,¹⁸⁵ TNF- α ,^{186,187} E-selectin,¹⁸⁸ and circulating progenitor cells,¹⁶³ lowers the risk of CRLM growth and recurrence.

Similar mechanisms to those identified in animal models have also been found in cases of post-hepatectomy and post-transplantation recurrence in patients.

Clinical evidence – liver resection

The main source of IRI in liver resection is vascular clamping. Vascular clamping through the Pringle manoeuvre refers to the selective occlusion of hepatic inflow from the liver hilum. The Pringle manoeuvre is commonly performed in liver surgery to reduce blood loss during parenchymal transection, in a fine balance between blood loss and IRI. Several authors have reported that an intermittent Pringle manoeuvre (*i.e.* short periods of occlusion with periods of reperfusion in between) does not increase the risk of recurrence of HCC^{189–191} or CRLMs.^{191,192} In a *post hoc* analysis of two randomized-controlled trials¹⁹³ on liver resection for HCC with vs. without an intermittent Pringle manoeuvre (88 patients per arm), better overall survival was

observed after an intermittent Pringle manoeuvre in patients with cirrhosis. Continuous, prolonged vascular clamping has instead been associated with worsened prognosis. In a retrospective analysis of 2,368 patients with HCC,¹⁹⁴ a Pringle manoeuvre lasting >15 min was associated with decreased overall and recurrence-free survival compared to one lasting <15 min or no Pringle manoeuvre. Nijkamp *et al.*¹⁹⁵ found that time to recurrence of CRLMs after resection was significantly shorter in patients with severe intraoperative ischaemia (*i.e.* continuous clamping for >20 min, or more than 3 cycles of \geq 15 min) compared to patients with no or minor ischaemia. Yamashita *et al.*,¹⁹⁶ in a retrospective analysis of 202 patients undergoing resection for CRLMs, reported worsened recurrence-free survival and cancer-specific survival in patients with higher remnant liver ischaemia. Similarly, in an analysis of 328 patients undergoing resection for HCC,¹⁹⁷ severe remnant liver ischaemia was associated with early recurrence within 6 and 12 months, and severe remnant ischaemia was a risk factor for diminished overall and recurrence-free survival.

Clinical evidence – liver transplantation

The liver graft experiences ischaemic damage at different time-points during procurement, storage, and implantation, with subsequent reperfusion injury once blood flow is restored inside the recipient. During the past decade, several retrospective studies have explored the correlation between the degree of IRI and post-transplant recurrence.

Factors influencing IRI and response to IRI in liver transplant are type of donor, quality of the graft, and comorbidities of the recipient. Regarding quality of the graft, we know for preclinical models that steatotic grafts have a lower tolerance for IRI. Severe steatosis of the graft has been associated with increased risk of tumour recurrence.^{20,198} The impact of donor age on post-transplant recurrence is controversial,^{199,200} but large analyses of the United Network for Organ Sharing (UNOS) database have found an association between older age and recurrence. Similarly, both increased body mass index^{23,201} and older age²⁰² of the recipient have been associated with increased HCC recurrence and poorer oncological outcomes.

While donor and recipient characteristics certainly play a role, the major driver of IRI is ischaemic time. Ischaemic damage varies greatly between donor types, with LDLT being associated with lower ischaemic times, DCDs being associated with the worst ischaemic damage due to WIT and procurement dynamics, and DBDs being somewhere in between.

As we will further describe in the next section, some authors have associated LDLT with increased HCC recurrence, however, this is thought to be related to the regenerative process that follows transplantation with a partial graft and a possible lack of patient selection due to reduced time on the waitlist and lower risk of waitlist dropout. No evidence on the role of ischaemia time in LDLT is currently available, nor would it be easy to dissect the contribution of IRI vs. regeneration.

Evidence from retrospective studies shows that ischaemia time leads to increased HCC recurrence after transplant with DBD grafts. Kornberg *et al.*²⁰³ found that CIT, WIT, and total ischaemia time were all prolonged in patients who developed HCC recurrence. WIT >50 min was independently associated with HCC recurrence at multivariate analysis, together with FDG (fluorodeoxyglucose)-avid HCC, alpha-fetoprotein levels of >400 IU/ml, and Milan-out status. Similar results were reported by Nagai *et al.*²¹ In their analysis on 391 patients undergoing transplant for

HCC, CIT of >10 h and WIT of >50 min were associated with increased recurrence, particularly within 1 year post-transplant. On multivariate analysis, both CIT and WIT remained significantly associated with recurrence, as did alpha-fetoprotein >200 ng/ml, Milan-out status, poor differentiation, and micro- and macrovascular invasion. Both Kornberg *et al.* and Nagai *et al.* identified a correlation between peak aspartate aminotransferase (AST) and prolonged ischaemia time. In a study by Grat *et al.*, AST was used as a surrogate for IRI, and patients with a peak AST $\geq 1,896$ U/L were found to have significantly lower recurrence-free survival. This correlation was more pronounced in patients who were classified as Milan-in, less so in patients who were Milan-out but Up-to-7-in, and not observed in patients beyond Up-to-7 criteria. This is in contrast with the findings by Kornberg *et al.* and Nagai *et al.*, who found the strongest correlation in patients with tumour-related prognostic features, FDG-PET avidity and microvascular invasion, respectively.

DCD grafts are considered extended criteria grafts that are associated with a higher risk of impaired outcomes compared to DBD donor livers; however, post-transplant outcomes with optimal DCD grafts (*i.e.*, young donors, short warm and cold ischaemia times) are comparable, if not superior to results seen with extended criteria DBDs.²⁰⁴ The most recent analysis of the UNOS database comparing DBD and DCD liver transplants in HCC recipients²⁰⁵ found no difference in HCC recurrence. While no survival difference was observed between DCD and DBD recipients with low risk for HCC recurrence, liver transplantation with DCD grafts was associated with lower recipient survival rates in patients at high risk of HCC recurrence.²⁰⁵ This further highlights the interplay between IRI levels, perioperative factors, and tumour biology. A previous analysis of the UNOS database²⁰ had found that the risk of HCC recurrence was increased in DCD grafts with prolonged WIT. Smaller single-centre studies^{206–208} have shown that good quality DCD grafts can lead to similar recurrence rates as seen with DBD grafts.

Box 3. Critical commentary on cancer recurrence and ischaemia-reperfusion injury.

Of the mechanisms highlighted in this review, IRI is at once the most detrimental and, at first glance, the easiest to target. Reducing IRI during liver resection only implies, after all, reducing Pringle manoeuvres and ensuring the proper vascularization of the remaining liver segments: two things that are easier said than done. Even more challenging is IRI in transplantation. In the real world, transplant centres have little control on the quality of the grafts they receive. They are often faced with the challenging situation of a suboptimal graft and a recipient who is not quite right for that graft but desperately needs a liver, especially those in intermediate urgency ranges. The severity of IRI is, however, not determined only by the quality of the graft, but cold ischaemia time plays a crucial role. The several logistical challenges of deceased donation limit any wiggle room for reducing ischaemic times per se, thus the most feasible option remains organ reconditioning, as we will see in the next paragraph.

Therapeutic options (Box 4)

Pharmacological interventions

Several therapeutic agents have been shown, in animal models, to reduce IRI, augment regeneration, or both.²⁰⁹ The most common mechanisms of action are either exerted on mitochondria, which represent the beginning of the IRI/regeneration cascade, or on downstream pathways, mainly regulation of the pro-

inflammatory response and reduction of apoptosis. Despite promising results in the preclinical setting, translation into clinical practice has been limited. Some clinical evidence exists on the benefits of S-adenosyl-L-methionine²¹⁰ and prostacyclin,²¹¹ but larger and more robust studies would be needed to support their use in standard practice.

Stem cells & extracellular vesicles

Mesenchymal stem cells (MSCs) and stem cell-derived extracellular vesicles (EVs) are novel strategies being investigated to modulate liver injury. The delivery of MSCs and MSC-derived EVs can inhibit inflammation, reduce apoptosis, necrosis and oxidative stress, and increase hepatocyte proliferation.²¹² Animal studies with mouse, rat and porcine models of IRI after hepatectomy and liver transplantation have shown that MSCs and MSC-derived EVs can improve liver regeneration and post-operative liver function,²¹³ reduce markers of IRI²¹⁴ and cellular death, and enhance ATP regeneration.²¹⁵ Clinical evidence on cirrhosis demonstrates improved liver function after treatment with MSCs.²¹⁶

Ischaemic conditioning

Ischaemic conditioning (IC) employs short periods of ischaemia followed by reperfusion to prime the target tissues, ameliorate their overall response and protect them from IRI. IC can be performed before (ischaemic *pre*-conditioning [IPC]), during (ischaemic *peri*-conditioning), or after prolonged ischaemia (ischaemic *post*-conditioning). It can then be applied directly to the target organ (*e.g.* the Pringle manoeuvre during hepatic resection) or indirectly (remote conditioning, *e.g.* putting a tourniquet around the patient's arm). The results of IC on IRI in preclinical cardiac and hepatic models have been encouraging. However, evidence from human studies is inconclusive, and most trials do not report long-term outcomes. Recent meta-analyses of RCTs failed to identify convincing evidence that remote IPC reduces IRI during liver resection²¹⁷ or transplantation,²¹⁸ while direct IPC has been shown to lower post-operative AST levels,²¹⁸ a possible surrogate of IRI, after transplantation. Well-designed studies with long-term endpoints are needed to confirm these preliminary results.

Machine perfusion

The standard graft preservation technique after procurement is static cold storage (SCS), *i.e.* an ice box. Recently, advances have been made in the field of organ preservation, including different types of machine perfusion. Machine perfusion represents a fascinating field, as it can potentially improve different aspects of the transplant process, from logistics, graft utilization, and viability assessment, to organ reconditioning and remodelling through the delivery of therapeutics during prolonged perfusion, *e.g.* defatting agents, senolytics, cell-based therapies, and gene modulation.

The two main machine perfusion approaches are hypothermic oxygenated perfusion (HOPE) and normothermic machine perfusion (NMP). Preservation with HOPE involves perfusion of the explanted graft, through either the portal vein alone or both the portal vein and the artery (dual HOPE), with a hypothermic solution containing a high concentration of oxygen (>60 kPa). Four RCTs are currently available and have shown that HOPE reduces early allograft dysfunction and leads to less overall and biliary complications, resulting in better post-transplant graft survival and lower retransplantation rates, compared to SCS in

extended criteria donor livers.^{219–221} By restoring oxygen in a hypothermic environment, HOPE reverses hypoxia and mitochondrial dysfunction and thus downregulates all the cascades that derive from them. The mitochondrial electron transport chain, in particular Complex I, resumes its activities, using oxygen for oxidative phosphorylation and ATP production. The byproducts of the TCA cycle that accumulate during hypoxia, which are responsible for massive ROS production during reperfusion, get degraded. This has a great effect in dampening IRI and all its consequences, as shown in Fig. 5. HOPE can also promote liver regeneration. By diminishing mitochondrial dysfunction, improving oxygenation through increased perfusion, and reducing inflammation, it allows mitochondria to provide sufficient energy to sustain the demands of regeneration.^{222,223}

Unlike HOPE, NMP is performed by perfusing the graft with a red blood cell-based solution at 35.5–37.5 °C. Since the liver metabolism is restored through perfusion with oxygen and nutrients, NMP allows for viability assessment through the measurement of IRI injury levels and functional parameters. A series of investigational parameters (i.e., bile production, lactate clearance, perfusate transaminases, pH, electrolytes, glucose, LDH, bicarbonate) were explored in a number of smaller case series lacking validation. While a standardized set of viability criteria has still not been established, NMP has proven successful in enabling the evaluation of the transplantability of grafts that would have been discarded based on clinical assessment alone.²²⁴ NMP has been shown to diminish early allograft dysfunction and ischaemic biliary complications compared to SCS.^{225,226} While NMP may also exert a dampening effect on IRI, its mechanisms of action are downstream relative to those of HOPE and mostly linked to a reduction in pro-inflammatory

cytokines, neutrophil infiltration and cell death during the late IRI phase, when a major part of graft injury has already occurred. No evidence exists so far on the effect of endischaemic NMP on cancer recurrence.

However, the significant reduction of IRI through the concept of “ischaemia-free” liver transplantation (IFLT), presented first by a pioneering group from China, could be of interest.²²⁷ For successful IFLT, the perfusion device is connected to the vessels during donor liver procurement, where the graft undergoes NMP with continuous perfusion during procurement, *ex situ* liver transfer to the recipient operating theatre and transplantation. Once the implantation is completed with revascularization of the entire liver graft, NMP is stopped.²²⁸ The same group performed a retrospective propensity score matched analysis,²²⁹ wherein 30 patients with HCC underwent IFLT and were compared to 85 patients undergoing liver transplantation after standard SCS. After matching, the two cohorts were similar with regards to demographic characteristics, pre-transplant AFP, tumours within Milan criteria, tumour differentiation, microvascular invasion, and pre-transplant treatments. Surrogates for IRI, such as AST and LDH on postoperative day 1, were diminished in the IFLT cohort. Recurrence-free survival at 1 and 3 years was higher in the IFLT group vs. the SCS group (92.2% and 86.7% vs. 88.1% and 53.6%, respectively, $p = 0.048$).

Meanwhile, Mueller *et al.*,²³⁰ in a retrospective multicentre analysis, matched 70 DCD grafts transplanted after endischaemic HOPE with 70 DBD and 70 DCD grafts transplanted after SCS. Post-transplant HCC recurrence was significantly lower in the HOPE-treated DCD group than in the unperfused DBD and DCD groups.

To date, HOPE is the only therapeutic modality that has been associated with reduction of IRI, promotion of regeneration, and

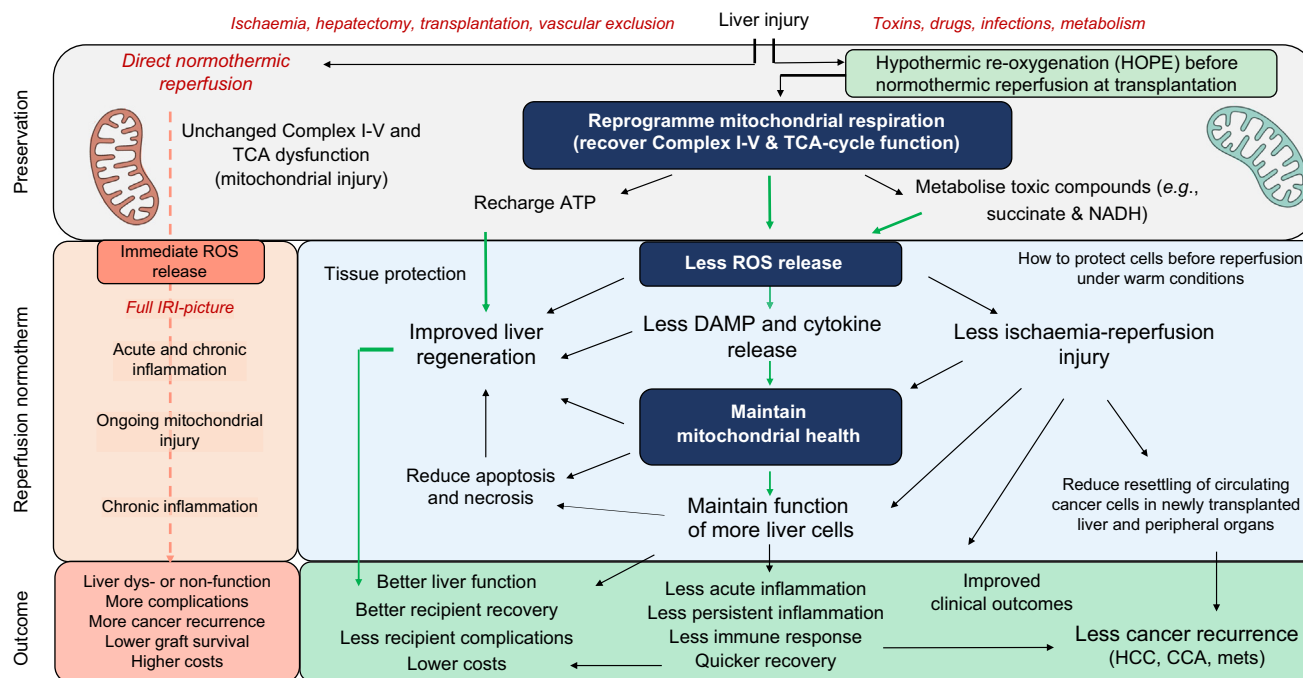


Fig. 5. How HOPE dampens liver injury during transplantation, leading to improved outcomes and reduced cancer recurrence. In case of direct normothermic reperfusion after static cold storage, the mitochondrial dysfunction caused by ischemia is not reversed, causing the full picture of IRI to develop as previously specified. Conversely, HOPE restores mitochondrial respiration and oxidative phosphorylation, leading to reduced release of reactive oxygen species. Less oxidative stress translated into less activation of downstream pathways and less inflammation, resulting in better postoperative liver function and better overall outcomes. CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma; HOPE, hypothermic oxygenated machine perfusion; ROS, reactive oxygen species.

reduction of post-transplant HCC recurrence. This has led to the design and recent initiation of two key clinical trials. The first is a multicentre RCT in Italy designed to confirm the previously described impact of HOPE on HCC recurrence after liver transplantation. The second is a study on the effect of HOPE in living donor grafts deemed too small with a high corresponding risk of small for size syndrome. This study will elegantly assess the combined effect of HOPE on IRI and regeneration, considering the key role of mitochondria in all liver cells. The results of these two trials are eagerly awaited.

Box 4. Critical commentary on therapeutic options to mitigate perioperative factors for recurrence.

The therapeutic strategies described in this section are either unlikely to have much impact (ischaemic conditioning and pharmaceutical agents) or unlikely to find broad utilization any time soon (stem cells); except, of course, for machine perfusion. The use of machine perfusion in the last decade has increased enormously, with organ preservation strategies now becoming standard of care in specific situations (e.g., normothermic regional perfusion followed by HOPE as standard protocol for DCDs in certain European countries). As utilization increases, our understanding of the benefits and pitfalls of different machine perfusion strategies is also increasing, but we are far away from unleashing their full potential. We are still figuring out basic aspects of machine perfusion, such as which strategy is better indicated, when to perform sequential perfusion, or when machine perfusion adds little to the overall outcome (or even if such cases exist). At the same time, more emphasis is being put on more refined features of machine perfusion, such as its impact on the immunological status, the future conditions of the graft and, of course, tumour recurrence. Although we have just scratched the surface of machine perfusion, we can envision wonderful things to come.

Conclusions & future perspectives

Prevention of tumour recurrence after curative-intent liver surgery remains largely an unmet need. Adequate patient selection, an operation conducted according to oncological principles, and the select use of adjuvant therapy are essential to reduce this risk; however, a deeper understanding of the mechanisms that favour recurrence may reveal novel potentially actionable targets. It is now evident that the degree of perioperative injury has an impact on oncological outcomes. This injury has several components, ranging from the simple act of squeezing the liver parenchyma to the dramatic haemodynamic changes that occur during transplantation. These components have interlacing roles that may exacerbate one another and eventually determine whether the liver will recover its function in the immediate postoperative period. As discussed, the striking activation of downstream pathways caused by perioperative injury not only affects the early postoperative period, but may create the perfect conditions for tumour engraftment while CTCs are released or activated in response to surgical manipulation and stress. Perioperative liver injury can be modulated by targeting different aspects, which may either diminish sources of damage (ischaemia-reperfusion, oxidative stress, immune activation, pro-inflammatory response) or boost liver function and regeneration. Therapeutic strategies that appear particularly promising are those addressing upstream pathways and exerting their effects at multiple levels in the liver injury cascade, such as stem cell therapy and hypothermic machine perfusion. Clinical studies are ongoing, and their results will hopefully shed light on ways to reduce perioperative liver injury and ameliorate short- and long-term outcomes that can be implemented in routine patient care.

Abbreviations

AST, aspartate aminotransferase; CIT, cold ischaemia time; CRLMs, colorectal liver metastases; CXCL10, C-X-C motif chemokine ligand 10; DAMP, damage-associated molecular patterns; DBD, donation after brain death; DCD, donation after cardiac death; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial-to-mesenchymal transition; ETC, electron transport chain; IC, ischaemic conditioning; IFLT, ischaemia-free liver transplantation; IL-, interleukin-; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HIF-1 α , hypoxia-inducible factor-1 α ; HOPE, hypothermic oxygenated machine perfusion; IPC, ischaemic pre-conditioning; IRI, ischaemia-reperfusion injury; MMPs, matrix metalloproteinases; MSCs, mesenchymal stem cells; NMP, normothermic machine perfusion; PVE, portal vein embolization; ROS, reactive oxygen species; SCS, static cold storage; TCA, tricarboxylic acid; TLR4, Toll-like receptor-4; TNF- α , tumour necrosis factor- α ; TSH, two-staged hepatectomy; UNOS, United Network for Organ Sharing; VEGF, vascular endothelial growth factor; WIT, warm ischaemia time.

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Conflict of interest

The authors declare no conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Concept and design, M.M. and A.S.; writing of article, M.M., A.S., S.Y., B.C., R.R., K.A.; review and editing of the article: all coauthors.; supervision, V.M., A.S.; All authors have read and agreed to the published version of the manuscript.

Supplementary data

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Author names in bold designate shared co-first authorship

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