

Review

Regulated cell death in organ transplantation: recent developments and mechanistic overview

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Organ transplantation is a definitive therapeutic option for patients with end-stage organ dysfunction and failure. Ischaemia–reperfusion (IR) injury is one of the leading causes of low graft utilization as it significantly increases the risk of primary graft dysfunction and acute rejection following transplantation. This risk is particularly high for organs obtained from donation after circulatory death (DCD) when compared with the organs from donation after brain death (DBD). IR injury exacerbates tissue damage via various mechanisms including the induction of regulated cell death. Regulated cell death and its consequences play critical roles in determining graft survival and function, thereby influencing the overall success of the transplant. Understanding the mechanisms underlying regulated cell death in IR injury is essential for developing therapeutic strategies to minimize tissue damage and improve clinical outcomes in organ transplantation. This review mainly discusses different types of regulated cell death and underlying mechanisms towards preventive cell death strategies in DBD and DCD organ transplantation in preclinical settings.

Keywords: organ transplantation, ischaemia–reperfusion, donation after circulatory death, donation after brain death, regulated cell death

Organ donation

Organ transplantation is one of the most significant advancements in medical history, offering many patients a final opportunity for survival. Since Dr Joseph Murray and his colleagues conducted the first successful kidney transplant in 1954 (Tan and Merchant, 2019), the shortage of grafts has remained one of the major challenges in transplantation, limiting the broader application of this vital therapeutic intervention (Lewis et al., 2021). The organ shortage is primarily due to the increasing demand for transplants and the associated limited availability of

suitable donated organs. In 2023 alone, ~88550 patients were awaiting a kidney transplant, while only 15900 kidney transplants were performed in the USA. Similarly, there were >10000 patients on the liver transplant waiting list, but only ~6000 liver transplants were performed. Additionally, the heart and lung graft shortage is getting worse, with ~2000 lung transplants and 3670 heart transplants performed annually in the USA, less than half of all patients on the waiting list (Colvin et al., 2024; Valapour et al., 2024).

Donation after brain death

Organ donors certified as brain-dead based on neurological criteria [referred to as donation after brain death (DBD) donors] maintain their circulatory and respiratory functions with the assistance of ventilatory support. During organ retrieval, circulation is stopped by clamping the aortic arch and draining the vena cava. Cold preservation solution is then flushed through the distal aorta to minimize anaerobic metabolism and reduce the risk of warm ischaemia (Saeb-Parsy et al., 2021). DBD donors

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significantly contribute to the organ supply in addressing the high demand for organs (Lewis et al., 2021). It is well-recognized that organs procured from DBD donors are of higher quality due to the continuation of donor circulation at the time of brain death declaration. This continuous supply of oxygen and nutrients to the organs until retrieval ensures a shorter duration of warm ischaemia, leading to improved organ quality and better transplant outcomes (McKeown et al., 2012). However, brain death is often accompanied by significant haemodynamic changes that can lead to organ injury (Lazzeri et al., 2021). During brainstem death, the body simultaneously releases catecholamines, producing a hyperdynamic cardiovascular response known as a 'catecholamine storm' (McKeown et al., 2012). This response increases cardiac output due to hypertension and bradycardia, leading to elevated blood pressure. Subsequently, there is a decrease in serum catecholamines and peripheral vascular resistance, which results in cardiovascular failure and hypovolaemia. These haemodynamic changes impair organ perfusion and contribute to organ injury. Meanwhile, the immune system can be severely disrupted during brain death, which leads to a rapid and uncontrolled release of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, tumour necrosis factor- α (TNF- α), and interferon-gamma (IFN- γ) (Hofmann et al., 2023). These cytokines not only trigger an aggressive immune response to increase the risk of acute rejection but also induce regulated cell death in multiple kinds of cells through caspase-8 activation (Deng et al., 2024). In addition, the systemic inflammation caused by cytokine release exacerbates ischaemia-reperfusion (IR) injury, resulting in a higher risk of primary graft dysfunction (PGD) and delayed graft function (DGF) (Mori et al., 2014).

Donation after circulatory death

Donation after circulatory death (DCD) is defined as retrieving donor organs for transplantation after death due to cardiac arrest. The utilization of DCD grafts significantly mitigates the ongoing organ shortage in many countries (Watson and Dark, 2012). Besides kidneys, organs such as the liver and lungs, which are more vulnerable to warm ischaemia, can also be successfully transplanted from DCD donors with reasonable survival rates (Le Dinh et al., 2012; Manara et al., 2012). However, a key challenge in the practice of DCD is the identification of suitable potential DCD donors and the minimization of warm ischaemic time (WIT) in a manner that is professional, ethical, and legally compliant (Manara et al., 2012).

Organs from DCD donors typically experience a longer duration of warm ischaemia compared to those from DBD donors. Warm ischaemia injury, which is most severe between cardiorespiratory arrest and the initiation of cold perfusion, actually begins during the decline of cardiorespiratory function. Consequently, in the case of DCD organs, the onset of functional WIT (fWIT) is now defined as when systolic arterial pressure drops to <50 mmHg, arterial oxygen saturation falls to <70%, or both (Kalisvaart et al., 2018). Compared to organs from DBD donors, prolonged fWIT in organs from DCD donors increases the risk of graft failure, DGF, and other ischaemic complications in

recipients. Therefore, it is crucial to explore strategies for mitigating warm ischaemic injury and identify effective treatments for achieving this goal.

Outcomes of organs from DBD and DCD donors

Compared to organs from DBD donors, DCD donor organs are generally believed to suffer significant damage due to prolonged WIT (Figure 1A), which impairs organ function and reduces the survival rate of transplanted organs (Elmer et al., 2022). In kidney transplantation, the use of organs from DCD donors is associated with a higher incidence of acute tubular necrosis, requiring post-transplant dialysis in >50% of recipients (Del Río et al., 2019; Mok et al., 2023). A retrospective review included 135644 recipients revealed that the risk of PGD and overall graft failure (1, 5, 10 years) was much higher in DCD kidneys than matched DBD kidneys, stratified by acute kidney injury stage (Lia et al., 2021). However, evidence also shows that transplanting DCD kidneys with WIT <2 h gets accepted long-term outcomes and has a lower risk of mortality when compared to patients on the waiting list (Scalea et al., 2017; Lia et al., 2021). DCD livers may carry a higher risk of primary non-function (PNF) and graft loss and are more prone to developing anastomotic and intrahepatic biliary strictures especially for donors in old age (Foley et al., 2011; Lee et al., 2014). On the other hand, DCD lung transplantation has shown acceptable outcomes comparable to those of DBD lung transplantation (Egan et al., 2021). DCD pancreas transplantation has been less extensively studied compared to other organs, primarily due to concerns about postoperative dysfunction and pancreatitis. However, previous data suggest that graft thrombosis rates and pancreas loss are similar between DCD and DBD pancreas transplants (Leemkuil et al., 2019; Richards et al., 2021).

IR injury in organ transplantation

Mechanisms of IR injury

The success of transplant surgery is directly influenced by IR injury, and there is a significant amount of ongoing research focused on developing therapeutic treatments to mitigate this damage. IR injury is characterized by organ damage occurring when blood supply is restored after a period of interruption. This interruption is inevitable, whether the graft is from DBD or DCD donors (Figure 1B). IR injury is a complex condition influenced by various factors, including hypoxia, oxidative stress, leukocyte extravasation, activation of cell death pathways, and immune responses (Kalogeris et al., 2012). Warm ischaemia occurs when a donor organ loses blood supply until it is perfused with a cold preservation solution. Cold ischaemia occurs when the organ is preserved at 4°C in a cold solution until blood flow is restored during implantation in the recipient. Reperfusion injury then starts when blood flow is reestablished to the organ in the recipient (Zhao et al., 2018).

During ischaemia, the lack of oxygen prevents the production of sufficient ATP through oxidative phosphorylation in mitochondria. As a result, organs cannot rely on the metabolism of sugar, lipids, and amino acids to maintain ATP level

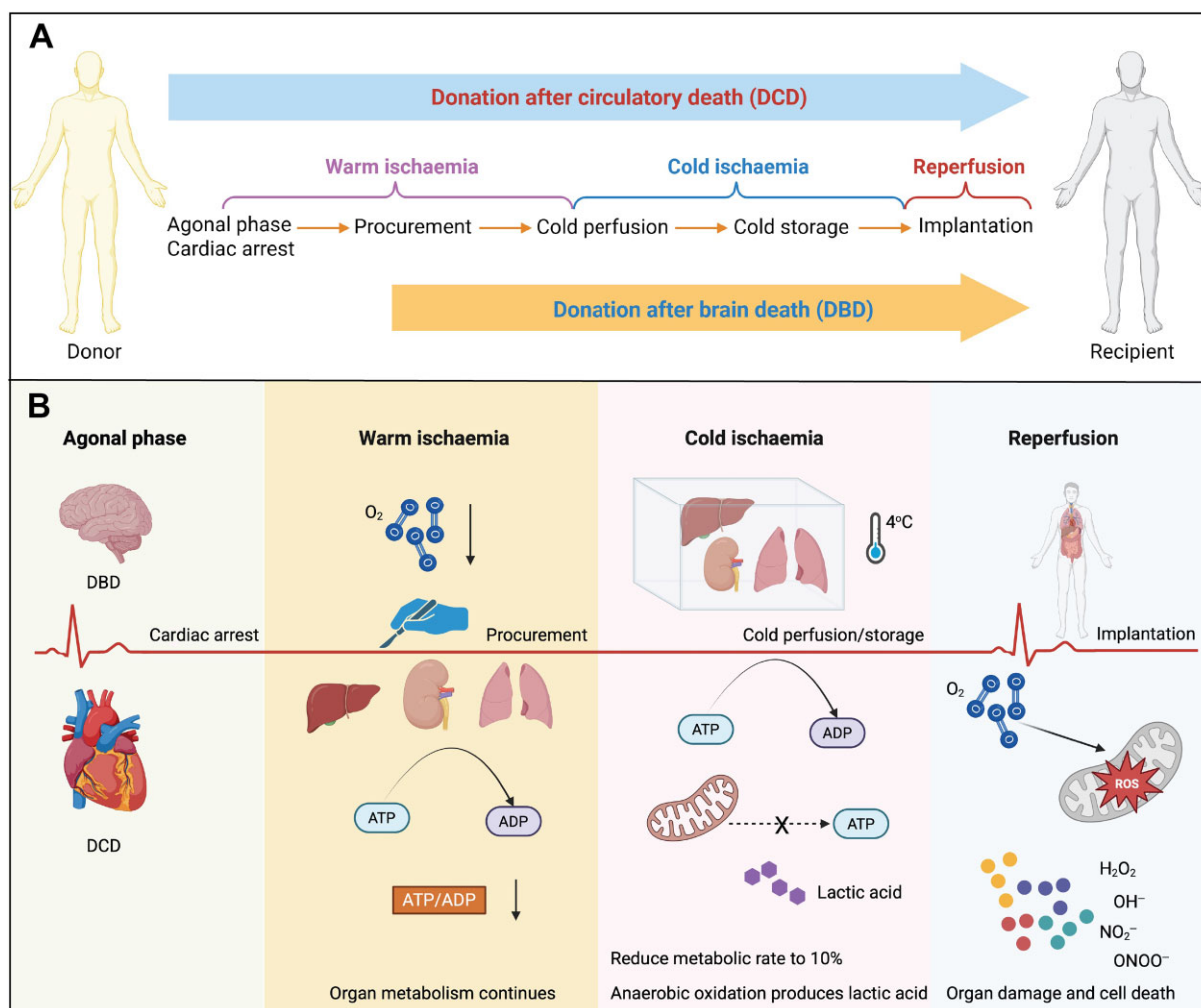


Figure 1 IR during organ donation and implantation. **(A)** Grafts from DCD donors experience longer WIT compared to those from DBD donors due to cardiorespiratory failure before cardiac arrest. **(B)** Grafts such as the kidney, liver, and lungs from DBD or DCD donors experience warm ischaemia when they lose blood flow until the end of perfusion with a cold preservation solution. These grafts are subsequently preserved in a cold preservation solution at 4°C and transported to the recipients. The low temperature during preservation reduces the metabolic rate and the demand for ATP by cells. Upon reperfusion, when blood flow is restored to the organ in the recipient, mitochondria produce an abundance of ROS. The imbalance between oxidative and antioxidative levels releases DAMPs from oxidative stressed cells, triggering sterile tissue inflammation. IR injury causes microcirculatory dysfunction, activation of T cells and macrophages, cell death, and inflammation. These processes increase the risk of post-reperfusion syndrome, graft dysfunction, PNF, acute rejection, and other complications, ultimately impacting the quality of life for the recipients. Image created with BioRender.com with permission.

(Saeb-Parsy et al., 2021). However, organs continue to consume ATP, rapidly reducing the intracellular ATP/ADP ratio. This reduction impairs cellular functions and can result in cell death. While cold storage significantly reduces ATP demand, a gradual decline in the ATP ratio still occurs, as anaerobic glycolysis remains necessary for cell survival and essential function, even at low temperatures (Saeb-Parsy et al., 2021). Consequently, the intracellular ATP level at the end of warm ischaemia is crucial in determining the organ subsequent tolerance to cold ischaemia. During reperfusion, the sudden influx of oxygen leads to rapid oxidation of succinate in mitochondria, generating reactive oxygen species (ROS) that cause significant damage to

lipids, proteins, DNA, and overall cellular function (Saeb-Parsy et al., 2021).

ROS have been identified as the primary elements responsible for IR injury. Superoxide and hydroxyl radicals are the main ROS in cells, and their cytotoxicity comes from lipid peroxidation-induced membrane damage and the formation of other free radicals that cause DNA damage (Zhao et al., 2017a). Previous studies demonstrated that increased ROS levels mediate neutrophil adhesion at the blood–endothelial cell interface, contributing to necrosis and initiating a feedback loop that exacerbates ROS production and cellular injury (Mittal et al., 2014). Furthermore, neutrophils generate significant amounts of

Table 1 Composition of cold preservation solutions.

Solutions	UW	EC	HTK	Celsior	Perfadex
K ⁺ (mEq/L)	125	115	10	15	6
Na ⁺ (mEq/L)	29	10	15	100	138
Cl ⁻ (mEq/L)	20	15	32	71	142
Ca ²⁺ (mEq/L)	/	/	0.015	0.25	/
Mg ²⁺ (mEq/L)	5	/	4	13	0.8
Buffer	Phosphate	Phosphate	Histidine	Histidine	Phosphate
Antioxidant	Glutathione, allopurinol	/	Mannitol, tryptophan, α -ketoglutarate	/	/
Glucose	/	180	/	5	10
Amino acids	/	/	Histidine, tryptophan	/	/

UW, University of Wisconsin; EC, Euro–Collins; HTK, histidine–tryptophan–ketoglutarate.

extracellular superoxide due to membrane-bound nicotinamide adenine dinucleotide phosphate oxidase. The superoxide is then converted to H₂O₂ through disproportionation. Excessive H₂O₂ production generates hypochlorous acid and hydroxyl radicals, further contributing to organ dysfunction (Zeng et al., 2019). ROS overproduction also impairs cellular calcium homeostasis by releasing intracellular calcium from the endoplasmic reticulum and increasing extracellular calcium entry through voltage-gated calcium channels or store-operated calcium entry channels. Increased calcium activates calcium-dependent enzymes, such as proteases and phospholipases, leading to cellular damage and dysfunction (Gorlach et al., 2015).

Static cold preservation

Preservation techniques, such as temperature control, modified preservation solutions, and perfusion settings, were developed to reduce cellular metabolism and provide organ protection against IR injury. Static cold storage is the current gold standard technique for organ preservation. Without blood flow, cells rapidly switch from aerobic to anaerobic metabolism. Anaerobic metabolism requires 19 times more glucose substrate to produce ATP than aerobic metabolism. This metabolic shift leads to the rapid depletion of intracellular glucose substrate and increases the accumulation of metabolites such as lactic acid. The purpose of cold organ preservation is to minimize these metabolic changes by reducing the metabolic rate to ~10% of normal body temperature (Heldmaier and Ruf, 1992). In clinical practice, various organ preservation solutions (Table 1) are currently used to mitigate IR injury (Guibert et al., 2011). To maintain intracellular pH, these solutions contain physiological buffers, such as phosphate or citrate, and large molecules, such as mannitol or raffinose, to preserve intravascular osmotic pressure and thus minimize cellular swelling.

Machine perfusion

With the increasing use of DCD donors and marginal donors, it has become essential to develop innovative organ preservation strategies. Among these, machine perfusion techniques have gained popularity due to their significant advantage in mitigating IR injury. Evidence suggests that normothermic local perfusion of organs from DCD donors can improve organ quality and reduce IR injury (Naranjo Gozalo et al., 2022). In the

DCD process, a double-balloon catheter is inserted through the femoral artery into the aorta to isolate the abdominal aorta, while blood is drained through the ipsilateral femoral vein. The catheter is subsequently connected to an extracorporeal membrane oxygenator circuit, which enables the perfusion of warm and oxygenated blood to the abdominal organs. This process supplies the organs with sufficient ATP through oxygenated blood, enhancing their ability to withstand subsequent cold ischaemia (Minambres et al., 2017). Preliminary studies have shown that this strategy improves the utilization and quality of the kidney, liver, and pancreas obtained from DCD donors (Hunt et al., 2022; Bekki et al., 2023).

The use of cold machine perfusion systems has garnered considerable attention. While static cold storage is simple and convenient for transporting organs from the donor to the recipient hospital, using a machine that circulates cold preservation solution may offer superior organ preservation. Continuous cold perfusion has the potential to greatly enhance the quality of transplanted organs by effectively flushing out accumulated metabolites from small capillaries, which is particularly beneficial for organs obtained from DCD donors (Chew et al., 2019). Recent studies demonstrated that this strategy significantly reduces the risk of PNF and shortens hospital stays for kidney transplant recipients (Moers et al., 2009; Mergental et al., 2021), as well as lowers the risk of complications such as non-anastomotic biliary strictures in liver transplant recipients (Mergental et al., 2021; Risbey et al., 2024).

Regulated cell death pathways in organ transplantation

Several regulated cell death pathways have been implicated in organ IR injury within the context of organ transplantation, including apoptosis, necroptosis, iron-dependent ferroptosis, and pyroptosis. The comparison of these regulated cell deaths is summarized in Table 2.

Apoptosis

There are two pathways that activate apoptosis: intrinsic and extrinsic pathways (Figure 2A). The extrinsic apoptotic pathway is usually triggered when death ligands, such as TNF- α and FASL, bind to their respective membrane death receptors such as tumour necrosis factor receptor (TNFR) and FAS. These interactions lead to the formation of signaling

Table 2 Comparison of apoptosis, necroptosis, ferroptosis, and pyroptosis.

	Apoptosis	Necroptosis	Ferroptosis	Pyroptosis
Morphological features	Plasma membrane blisters, cell shrinkage, nuclear condensation and chromatin margination, apoptotic bodies	Cell swelling, plasma membrane disruption, cytoplasmic vacuolisation, mitochondrial damage	Shrunk and electron-dense mitochondria, increased mitochondrial membrane density, decreased mitochondrial volume, plasma membrane disruption	Cell swelling, plasma membrane disruption, cell lysis, cytoplasmic vacuolisation, formation of pyroptotic bodies (inflammatory bodies)
Biochemical features	Caspase activation	RIP-regulated, MLKL phosphorylation	Iron metabolism	Inflammasome activation, caspase-1 and GSDMD activation
Activation conditions	DNA damage, ROS overload, death receptor activation, infection and immune response, developmental process	Severe oxidative stress, Ca ²⁺ overload, death receptor activation	Depletion of GSH, dysfunction of GPX4, iron accumulation, and lipid peroxidation	LPS, NLRP3 and other inflammasome bodies
Key genes	Caspase-3, Bcl-2, Bax, Fas	Ripk1, Ripk3, MLKL, CYPD, LEF1	GPX4, SCL7A11, P53, FSP1, ACSL4, VDAC2/3, TFR1, FTH1, FTL	Caspase-1, IL-1, IL-18, GSDMD
Relevant diseases	Cancer, autoimmune disease	Infection, neurodegeneration disease, inflammatory disease, trauma, toxins	Cerebral stroke, cancer, acute kidney/liver injury, neurodegeneration disease	Infection, inflammation, neurodegeneration disease, cardiovascular disease, cancer

complexes, including complex I and the death-inducing signaling complex (DISC), which activates caspase-8 and may lead to mitochondrial damage (Lucas-Ruiz et al., 2022; Ai et al., 2024). On the other hand, intracellular stress usually activates the intrinsic apoptotic pathway, which is mediated by mitochondrial translocation of Bcl-2-associated X protein (BAX) and Bcl-2 antagonist killer 1 (BAK), culminating in mitochondrial outer membrane permeabilization (MOMP) (Elmore, 2007; Ai et al., 2024). Both the intrinsic and extrinsic apoptosis pathways ultimately rely on activating the executioner proteins caspase-3 and caspase-7. Caspase-3 can be cleaved to generate p12 and p17 subunits, which initiate DNA fragmentation and degradation of the cytoskeleton and nucleoproteins (Elmore, 2007).

Caspase activation is increased in models of kidney, liver, and lung IR injury (Detelich et al., 2018; Lorente et al., 2019; Liu et al., 2021, 2024). In kidney transplantation, administering caspase-3 siRNA intravenously or through cold perfusion has been shown to reduce renal cell death and inflammation (Yang et al., 2014; Li et al., 2021). Similarly, inhibiting caspase-3 before IR in lung transplantation has been found to improve lung function and decrease cell death in rats (Quadri et al., 2005; Li et al., 2018). In rat liver transplants, the addition of DEVD-FMK to the preservation solution, which inhibits caspase-3 and caspase-7, has been demonstrated to reduce microvascular injury and improve the survival of transplanted organs (Mueller et al., 2004). Additionally, studies reported that administering a pan-caspase inhibitor during cold perfusion enhances liver function in rats (Baskin-Bey et al., 2007; Bral et al., 2019; Raigani et al., 2022). Supplementing preservation solutions with pan-caspase inhibitors has yielded similar benefits for preserving human livers

(Baskin-Bey et al., 2007). In heart transplantation, administering a caspase-3 inhibitor to either the donor or recipient before transplantation can reduce coronary artery vasculopathy in mice (Tanaka et al., 2004; Skorić et al., 2014). These findings suggest that caspase inhibition may reduce apoptosis in transplanted organs.

Necroptosis

Necroptosis is a form of regulated cell death characterized by plasma membrane rupture and the release of cellular contents. Necroptosis plays a role in post-transplant graft dysfunction, including DGF and PNF. Necroptosis is typically initiated following activation of various cell surface death receptors such as FAS, TNFR, and toll-like receptors (TLRs), particularly when caspase-8 is inactive or inhibited, allowing receptor-interacting protein kinase 1 (RIPK1) to initiate downstream signaling (Figure 2B; Zhao et al., 2014b; Galluzzi et al., 2017; Dhuriya and Sharma, 2018; Ai et al., 2024; Deng et al., 2024). Upon stimulation by signals such as TNF- α and virus, cell surface receptors are activated, leading to the binding and activation of RIPK1, TRIF, and ZBP1, respectively, all of which contain the key necroptotic signaling protein domain called RIP homotypic interaction motif (RHIM) (Park et al., 2014; van Loo and Bertrand, 2023). When the activation of caspase-8 is inhibited, the RHIM domains of RIPK1 and RIPK3 interact, leading to the phosphorylation of RIPK3, which subsequently phosphorylates the mixed lineage kinase domain-like (MLKL) protein. The N-terminal 4-helix bundle domain of phosphorylated MLKL then binds to liposomes containing phosphatidylinositol phosphates, causing the rupture of membranes and releasing inflammatory factors and danger-associated molecular patterns (DAMPs) (Wang et al., 2014; Miyake et al., 2019; Garnish et al., 2021).

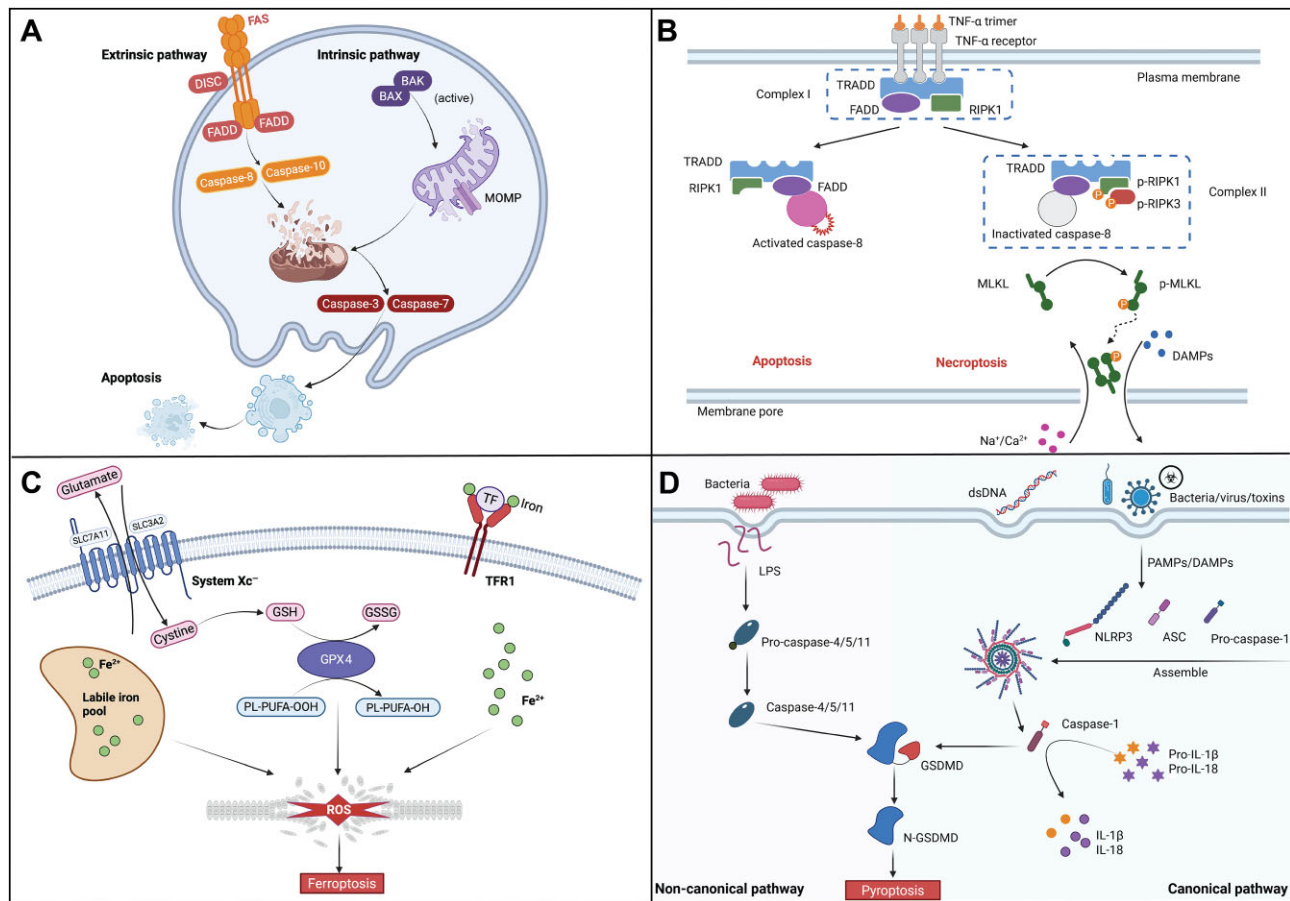


Figure 2 Molecular mechanism of regulated cell death. **(A)** Apoptosis. Cell injury (such as IR injury) activates the instinct apoptosis pathway by activating BAX and BAK to form MOMP. The extrinsic pathway is triggered by the activation of death receptors (e.g. FAS/FASL) to form a DISC and then active caspase-8/10. The activation of caspase-7 and caspase-3 ultimately initiates both instinct and extinct pathways. **(B)** Necroptosis. Upon the stimulation of the TNF- α primer, the TNF- α receptor combines with RIPK1, FADD, and TRADD, forming complex I. Then, FADD binds to the activated caspase-8, resulting in apoptosis. When the caspase-8 is inhibited, RIPK1 and RIPK3 combine and are phosphorylated, forming complex II. This complex promotes the oligomerization of MLKL by phosphorylating it. The oligomeric form of p-MLKL then transfers towards the plasma membrane from the cytosol, forming membrane pores, ultimately leading to $\text{Na}^+/\text{Ca}^{2+}$ influx and the release of DAMPs. **(C)** Ferroptosis. Transferrin (TF) imports Fe^{3+} from the extracellular environment to the cytoplasm through TFR1 recognition, and then Fe^{3+} is converted to Fe^{2+} through Fenton reaction. The excess iron catalyzes the production of ROS that contributes to lipid peroxidation within the cell membrane. When system Xc^- is inhibited, the source of cystine is reduced, and then the amount of GSH decreases. GSH depletion causes the inactivation of GPX4, which results in the accumulation of lipid peroxides and subsequent ferroptosis. **(D)** Pyroptosis. Right: the canonical pyroptosis signalling pathway. PAMPs and DAMPs active NLRP3 and then caspase-1, and caspase-1 can be directly activated by dsDNA. Activated caspase-1 promotes the release of IL-1 β and IL-18 and cleaves GSDMD. The N-terminus of GSDMD (N-GSDMD) induces pyroptosis and damages the plasma membrane. Left: the non-canonical pyroptosis signalling pathway. Bacterial products such as LPS directly activate murine caspase-11 (human caspase-4/5), which leads to the leakage of intracellular ATP, mediates the NLRP3/caspase-1 signalling pathway, and then induces plasma membrane pores. Image created with BioRender.com with permission.

A previous study demonstrated that mice with knockout of the *Ripk3* gene (*Ripk3*^{-/-}) exhibited resistance to renal IR injury (Lau et al., 2013). *Ripk3*^{-/-} kidneys showed lower histological injury scores, reduced neutrophil infiltration, decreased fibrosis, and less vascular damage compared to the control transplanted kidneys (Lau et al., 2013). Moreover, mice receiving *Ripk3*^{-/-} kidneys experienced prolonged survival without experiencing acute or chronic rejection (Lau et al., 2013). In liver transplantation, patients who develop early allograft dysfunction (EAD)

after liver transplantation exhibited significantly higher p-MLKL expression in liver grafts compared to those who did not develop EAD (Lee et al., 2016; Mazilescu et al., 2021). Furthermore, liver grafts with high p-MLKL expression had transaminitis and elevated lactate dehydrogenase at 24 h after transplantation (Wei et al., 2021). In lung transplantation studies, supplementing a necroptosis inhibitor (Nec-1) to cold preservation solution results in a significant reduction in pulmonary oedema and necrosis following transplantation in rats (Wang et al., 2019).

Although off-target effects and poor pharmacokinetics limited the clinical translation of necroptosis inhibitor Nec-1 (Cao and Mu, 2021), the new generation of RIPK1 inhibitor, GSK2982772, has advanced into clinical trials, demonstrating its acceptable safety profiles in reducing post-transplant graft injury.

Ferroptosis

Ferroptosis is a form of regulated cell death that is iron-dependent and driven by the peroxidation of membrane phospholipids, particularly those containing polyunsaturated fatty acids (PUFA-PLs) (Li et al., 2020a; Yan et al., 2021; Ai et al., 2024). The biosynthesis of PUFA-PLs is facilitated by the enzymes ACSL4 and LPCAT3, which incorporate PUFA-PLs into membrane phospholipids (Figure 2C). These PUFA-PLs are highly susceptible to oxidative modification by enzymes such as lipoxygenases, cytochrome P450 oxidoreductase, and cytochrome b5 reductase, leading to the generation of lipid peroxides that trigger ferroptosis. The Fenton reaction further amplifies lipid peroxidation through generating ROS in an iron-catalyzed process (Chen et al., 2020). In this reaction, ferrous iron (Fe^{2+}) reacts with hydrogen peroxide (H_2O_2) to generate highly reactive hydroxyl radicals ($\cdot\text{OH}$) and ferric iron (Fe^{3+}). These hydroxyl radicals aggressively oxidize PUFA-PLs, triggering a chain reaction that destabilizes cell membranes and ultimately leads to cell death. In contrast, the synthesis of monounsaturated fatty acid-containing phospholipids, catalyzed by SCD1, ACSL3, and MBOAT1/2, competes with PUFA-PL synthesis and acts as a protective mechanism by inhibiting ferroptosis.

Ferroptosis is also tightly regulated by several antioxidant defence systems. The system Xc^- cystine/glutamate antiporter imports extracellular cystine, a critical precursor for glutathione (GSH) synthesis. GSH, in turn, is required by glutathione peroxidase 4 (GPX4) to detoxify lipid hydroperoxides (Bridges et al., 2012; Tang et al., 2021). Impairment of GSH synthesis or depletion of GPX4 activity leads to lipid peroxide accumulation and the induction of ferroptosis (Forcina and Dixon, 2019). In addition to the GPX4–GSH axis, other antioxidant mechanisms serve to counteract ferroptosis. GTP cyclohydrolase 1 synthesizes tetrahydrobiopterin and endogenous antioxidant, while ferroptosis suppressor protein 1 (FSP1) reduces coenzyme Q10 (CoQ10) and vitamin K (VK) to their active antioxidant forms, CoQ10H_2 and VKH_2 , respectively. Within mitochondria, dihydroorotate dehydrogenase also contributes to antioxidant defence by reducing CoQ10 , further preventing lipid peroxidation in this organelle.

Recent studies suggest that certain components related to ferroptosis, such as GPX4 and the system Xc^- transporter, may be involved in the outcomes of transplanted kidneys. Animal models have demonstrated that inhibiting ferroptosis through modulating GPX4 or system Xc^- transporter enhances graft survival (Liao et al., 2022; Tang et al., 2023). Similarly, in liver transplantation, preoperative serum ferritin levels are an independent risk factor for recipients, with ferroptosis playing a significant role in the development of hepatic IR injury (Yamada et al., 2020; Wu et al., 2021). Although evidence regarding fer-

roptosis in lung transplantation is limited, it has been suggested that IR-induced ferroptosis in the early stages triggers excessive inflammation, leading to lung injury in animal models (Liu et al., 2023). These findings underscore the potential involvement of ferroptosis in organ transplantation and highlight the need for further research to clarify the underlying mechanisms. Ferroptosis inhibitors such as ferrostatin-1 and liproxstatin-1 were shown to be protective in renal and hepatic IR models (Miotto et al., 2020; Yamada et al., 2020; Shi et al., 2024; Chen et al., 2024c), although human data are currently lacking.

Pyroptosis and NLRP3 inflammasome

Pyroptosis is a highly inflammatory form of regulated cell death mediated by the gasdermin (GSDM) family of proteins (Bergsbaken et al., 2009; Ai et al., 2024). The key molecular machinery involved in pyroptosis is the NLRP3 inflammasome, a multi-protein complex that detects cellular stress and initiates the inflammatory cascade (de Vasconcelos and Lamkanfi, 2020). Two main pathways, the canonical and non-canonical pathways, have been identified for pyroptosis (Figure 2D). In the canonical pathway, immune cells detect pathogen-associated molecular patterns (PAMPs) or DAMPs via pattern recognition receptors (PRRs) that are also known as inflammasome sensors, including NLRP1, NLRP3, NLRC4, AIM2, and pyrin. Upon activation, these sensors assemble with pro-caspase-1 and the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) to form the inflammasome complex, which activates caspase-1 (Lu et al., 2014; Kelley et al., 2019). Activated caspase-1 cleaves gasdermin D (GSDMD), a critical effector protein in pyroptosis. Cleaved GSDMD forms pores in the plasma membrane, resulting in cellular swelling, membrane rupture, and intracellular contents release (Xia et al., 2021). Inflammatory cytokines, such as $\text{IL-1}\beta$ and IL-18 , promote inflammation and contribute to the inflammatory response associated with pyroptosis (He et al., 2015; Lei et al., 2018). In the non-canonical pyroptotic pathway, human caspase-4 and caspase-5 (or murine caspase-11) are directly activated, leading to a series of downstream events resulting in pyroptotic cell death (Swanson et al., 2019). Additionally, alternative pyroptotic pathways have been reported. These include caspase-3- and caspase-8-mediated pathways, as well as granzyme-mediated cleavage of gasdermins (GSDME or GSDMB) (Ai et al., 2024). However, the role of these non-canonical mechanisms in organ transplantation remains largely unexplored and warrants further investigation.

There is evidence suggesting that pyroptosis is involved in inflammation and organ damage during IR. Under ischaemic conditions, there is often an increase in ROS production and mitochondrial dysfunction. ROS can directly activate the NLRP3 inflammasome, further contributing to the induction of pyroptosis during reperfusion. Additionally, mitochondrial dysfunction can lead to the release of mitochondrial DNA, which acts as a danger signal and triggers inflammasome activation. Although there is limited clinical research on the role of pyroptosis in organ transplantation, a previous study conducted in rodents suggested that the expression of pyroptosis biomarkers, NLRP3

and caspase-1, was higher in donated hearts from DCD mice than in the hearts from DBD mice after warm ischaemia, and the elevation was reduced when donors were treated with an NLRP3 inhibitor (Lu et al., 2021). Furthermore, increased expression of the NLRP3 inflammasome, driven by the HMGB1–TLR4 pathway, has also been implicated in kidney injury and sterile inflammation in high-risk donor kidneys (Florim et al., 2020). Interestingly, in a rat lung graft normothermic perfusion model, it was observed that circulating leukocytes derived from donor lungs played a crucial role in impairing the quality of lung grafts through the activation of pyroptosis. When a caspase-1 inhibitor was administered to inhibit leukocyte pyroptosis, the release of immunological molecules and pro-inflammatory cytokines was significantly reduced, which, in turn, mitigated IR injury and lowered the incidence of PGD following lung transplantation (Noda et al., 2017). Currently, the caspase inhibitors such as VX-765 and GS-9450 have been well tolerated in phase I/II trials and have been shown to reduce IL-1 β release (Dhani et al., 2021). These findings highlight the potential of targeting pyroptosis as a therapeutic strategy to reduce organ injury.

Notably, the activation of the NLRP3 inflammasome associated with pyroptosis can also be triggered during necroptosis and ferroptosis. Recent findings indicated that certain necroptosis-related proteins trigger the activation of the NLRP3 inflammasome (Silk et al., 2017; Wang et al., 2023). For example, the necroptotic executor, MLKL, may act as an intrinsic stimulator of the NLRP3 inflammasome, activating NLRP3 in a GSDMD-independent manner resulting in the subsequent secretion of inflammatory cytokines (Huang et al., 2021). Additionally, inhibition of caspase-1 resulted in direct activation of NLRP3 by MLKL via K⁺ efflux in response to intracellular dsDNA (Conos et al., 2017; Liang et al., 2020). Previous studies have shown that MLKL also regulates the NLRP3 inflammasome through directly activating the adapter protein ASC or NF- κ B transcription factor. Therefore, the NLRP3 inflammasome may be involved in the inflammatory response triggered by necroptosis (Chao et al., 2017). The inhibitory effect of GPX4 on NLRP3 inflammasome activation has been demonstrated through GSDMD and caspase-11-dependent mechanisms (Kang et al., 2018), suggesting that GPX4 may have an inhibitory influence on pyroptosis. Moreover, the NLRP3 inflammasome activation may also induce several types of regulated cell death, such as ferroptosis and necroptosis, leading to inflammatory and immunological reactions (He et al., 2016; Huang et al., 2021). The process of stimulation and activation within this cycle is believed to substantially impact the progression of acute organ damage and dysfunction after transplantation.

Other regulated cell death and organ transplantation

There are several other regulated cell death pathways distinct from the aforementioned pathways, such as parthanatos and autophagy-dependent cell death. Parthanatos is a non-apoptotic regulated cell death pathway characterized by hyperactivation of poly ADP-ribose (PAR) polymerase 1 (PARP-1) (Fatokun et al., 2014; Zhao et al., 2015b; Silk et al., 2017).

During organ transplantation, the accumulation of ROS can directly damage DNA or cause the release of calcium from the endoplasmic reticulum, both of which lead to the activation of PARP-1 (Zhao et al., 2015b; Wang et al., 2024). This process is closely linked to parthanatos, which shares a common aetiology with other cell death pathways during organ transplantation.

PARP-1 is typically activated in response to DNA damage to facilitate repair, but when damage is extensive, such as during IR injury, hyperactivation of PARP-1 can lead to cell death (Li et al., 2020b). PARP-1 induces cell death by synthesizing PAR using NAD⁺ as a substrate, depleting intracellular NAD⁺ and rendering the cell unable to use it for glycolysis. This reaction itself expends ATP, further depleting the cell of its ATP sources. The synthesized PAR translocates apoptosis-inducing factor (AIF) from the mitochondria into the nucleus, which induces chromatin condensation and fragmentation (Daugas et al., 2000). This reaction also consumes ATP, further depleting the energy reserves of cells. The resulting ATP deficiency and AIF translocation lead to necrotic, non-apoptotic cell death.

Notably, PARP-1 is inactivated when cleaved by caspase-3, distinguishing parthanatos from apoptosis as distinct modes of cell death (Los et al., 2002). Despite its necrotic characteristics, parthanatos is a regulated form of cell death with well-defined initiation pathways and biomarkers. PARP-1 has been implicated in cardiac allograft rejection (Chen et al., 2021) and is associated with inducing distant pulmonary injury following ischaemic renal allografts (Zhao et al., 2015b). Interestingly, the common anaesthetic, propofol, has been shown to protect against cerebral IR injury by reducing ROS-induced calcium release during parthanatos (Zhong et al., 2018), suggesting potential benefits for its use during transplantation. These findings support the idea that targeting parthanatos could be a promising therapeutic strategy for improving the outcomes of organ transplants, warranting further research to fully understand its role in this context.

Autophagy-dependent cell death may also be related to IR injury in the context of organ transplantation. Autophagy normally serves to maintain cellular homeostasis through conserved degradation of unnecessary or dysfunctional intracellular proteins. However, when hyperactivated, autophagy can lead to the degradation of essential proteins, ultimately causing cell death. This has been observed in cardiomyocytes, where excessive autophagy stimulates cell death during IR injury (Valentim et al., 2006). Conversely, autophagy has also been shown to promote cell survival, particularly through degradation of dysfunctional mitochondria in a process known as mitophagy (Lin et al., 2024). Mitophagy helps to prevent the accumulation of cytotoxic oxidants, thereby reducing ROS-mediated cell death mechanisms such as ferroptosis and parthanatos (Su et al., 2023). The dual nature of autophagy, acting as both a promoter and an inhibitor of cell viability, makes it a 'double-edged sword' during IR injury (Ma et al., 2015). Given its complex roles, further exploration of autophagy in the context of organ transplantation is essential to understand how

it might be harnessed or modulated to improve transplant outcomes.

Overlap and crosstalk between regulated cell death pathways

Despite the identification of distinct regulated cell death pathways, numerous studies showed that inhibiting apoptosis, necroptosis, ferroptosis, or pyroptosis can each independently reduce graft injury in similar models, particularly in IR injury. This apparent overlap reflects the complex crosstalk and compensatory mechanisms among regulated cell death pathways. For instance, pyroptosis and apoptosis share the same upstream signal caspase-3; inhibition of caspase-8 can shift cell death from apoptosis to necroptosis, while oxidative stress can simultaneously activate both ferroptosis and apoptosis (Tang et al., 2019; Ai et al., 2024). In addition, the release of DAMPs during necroptosis stimulates dendritic cells and macrophages through PRRs, promoting innate immune activation and enhancing antigen presentation to alloreactive T cells (Kaczmarek et al., 2013). Similarly, immune cells detect DAMPs via PRRs activating pyroptosis, resulting in the release of IL-1 β and IL-18, which recruit neutrophils, promote Th1/Th17 responses, and amplify the alloimmune cascade (Kelley et al., 2019; Zheng et al., 2020). Both pathways serve as amplifiers of inflammation and aggravate IR injury, promote acute rejection, and contribute to the development of graft dysfunction. Moreover, ZBP1 has emerged as a sensor of nucleic acid stress and a central mediator of PANoptosis, a term describing the coordinated activation of pyroptosis, apoptosis, and necroptosis (Malireddi et al., 2019). ZBP1 contains a RHIM that allows it to interact with RIPK3, promoting necroptosis. Simultaneously, ZBP1 can facilitate inflammasome activation, leading to pyroptosis, and also engage caspase-8, driving apoptosis. This integration of pathways positions ZBP1 as a master regulator of inflammatory cell death (Malireddi et al., 2019). The contribution of ZBP1-driven PANoptosis in transplantation remains underexplored, but its known roles in systemic inflammation and IR models suggest that it may be a critical determinant of graft viability and immune activation.

Current studies showed that different cell types within the graft may preferentially activate specific regulated cell death mechanisms, and these may change dynamically over time following injury (Chen et al., 2023). Furthermore, the release of DAMPs from one death pathway can initiate inflammatory cascades that promote other forms of regulated cell death. Notably, pan-caspase inhibitors broadly suppress apoptosis and pyroptosis but may impair immune surveillance and promote alternative forms of cell death such as necroptosis (Vandenabeele et al., 2006). In contrast, specific caspase inhibitors allow for pathway-specific modulation might be with fewer adverse effects and better preservation of homeostatic cell turnover, which highlight the therapeutic potential of targeting regulated cell death pathway. These observations highlight the redundancy and plasticity of cell death programmes in transplantation and underscore the need for integrated approaches that target multiple regulated cell death pathways or are tailored to specific cell types and post-transplant time course. Moreover, there is an urgent need for

further in-depth research to determine whether a combination of inhibitors targeting multiple regulated cell death pathways provides a more effective strategy to reduce IR injury in organ transplantation.

Xenotransplantation and its implications for regulated cell death in organ transplantation

Xenotransplantation has made significant progress in clinical applications, with several patients having received pig-derived organs, including kidneys, hearts, and livers (Jaffe et al., 2025). However, unique challenges, including hyperacute rejection, vascular incompatibility, and enhanced innate immune activation, remain critical obstacles to xenograft survival. The massive inflammatory response towards a species-incompatible organ is driven by surface antigens, human leukocyte antigen incompatibilities, and necroinflammation (Arabi et al., 2023; Jaffe et al., 2025). In future, the contribution of necroinflammation will be of major interest in this field because of the comparably straightforward possibilities of inhibiting certain regulated cell death in grafts derived from pigs. One hypothesis yet to be tested in this field is that xenogeneic organs undergoing regulated cell death may trigger a more severe immune response compared to allogeneic organs. Nevertheless, gene-editing technologies such as CRISPR-Cas9 should enable control over this issue within a reasonable timeframe (Meier et al., 2018; Ladowski et al., 2019). The feasibility of inhibiting specific cell death pathways in pigs through genetic engineering is high, making the role of necroinflammation in xenotransplant immune responses to be a key focus. For instance, if necroptosis is identified as the dominant pathway, RIPK3 or MLKL may potentially be added to the list of deleted proteins in donor pigs, thereby reducing their immunogenicity.

Organ preservation strategies against IR injury and regulated cell death

Anti-ischaemic drugs

Anti-ischaemic drugs for organ preservation and their effectiveness in reducing IR injury have been analysed. Trimetazidine (TMZ) has emerged as a promising anti-ischaemic agent (Hauet et al., 2000). Studies demonstrated that the addition of TMZ to preservation solutions significantly improved kidney graft function and reduced IR-related damage, such as fibrosis and proteinuria. When cold storage solutions for kidneys were supplemented with TMZ and polyethylene glycol (PEG), better outcomes were observed in terms of creatinine clearance, reduced interstitial inflammation, and enhanced tissue protection compared to solutions without these agents (Faure et al., 2004). Pharmacological cocktail containing metformin, bucladesine, and cyclosporine A in cold-preserved livers after cardiac arrest significantly reduced hepatic enzyme release and lipid peroxidation, while mitigating inflammation and cell death during reperfusion in rats (Malkus et al., 2024). In addition, the use of sodium thiosulfate (STS) and Hem pure (a blood substitute) in kidney preservation solutions at 10°C demonstrated improved early post-transplant graft function, including enhanced

glomerular filtration rate and reduced tissue damage, though long-term outcomes did not reach statistical significance (Abou Taka et al., 2024). ADD10 improved early renal graft function by enhancing cell viability at 10°C, though long-term benefits were limited (Cassim et al., 2022). Glutathione with PEG35 preserved ATP levels, reduced oxidative stress, and maintained mitochondrial integrity in fatty liver grafts (Bardallo et al., 2022). Mitoquinone decreased oxidative stress, apoptosis, and kidney injury, improving mitochondrial function in DCD kidney grafts (Radajewska et al., 2023).

Proteasome inhibitors

Proteasome inhibitors, particularly bortezomib and epoxomicin, have shown significant potential in improving organ preservation during transplantation by alleviating IR injury. When bortezomib was added to the Institut George Lopez (IGL-1) preservation solution, it effectively reduced liver damage in steatotic livers and enhanced graft function by activating the AMPK and Akt/mTOR pathways, while this protective effect was reduced when an AMPK inhibitor was introduced, highlighting the critical role of this pathway (Bejaoui et al., 2014; Hamada et al., 2018). Similarly, epoxomicin, a specific proteasome inhibitor, demonstrated its effectiveness in reducing myocardial injury and edema in ischaemic hearts during cold storage by inhibiting a subset of proteasomes activated as ATP levels declined (Geng et al., 2009). These findings suggest that proteasome inhibition could be a promising strategy for enhancing organ preservation and reducing IR injury during transplantation.

Hormone drugs

Hormone drugs have emerged as critical agents in enhancing organ preservation strategies, significantly improving transplantation outcomes. Melatonin, for instance, has demonstrated profound efficacy in mitigating renal ischaemic injury, evidenced by its ability to reduce lactate dehydrogenase activity and improve histopathological scores in both kidney and liver grafts (Zaouali et al., 2011; Coskun et al., 2022). Similarly, the incorporation of PEG and pan-caspase inhibitors such as emricasan has been shown to bolster hepatocyte viability by counteracting cold-induced cellular damage during preservation, yielding promising results in both isolated hepatocyte models and *in vivo* pig kidney transplantation (Chen et al., 2024a). ECPEG (low K⁺ PEG) improved kidney function, reduced fibrosis, immune infiltration, and proteinuria, especially with TMZ (Faure et al., 2004). PEG-flushed kidneys showed less tubular damage, lower immune infiltration, better function, and delayed fibrosis. Iloprost in lungs improved alveolar and endothelial integrity, reduced surfactant loss, and limited tissue damage during storage (Hauet et al., 2002). Thyroid hormones have also been identified as potent cytoprotective agents, enhancing mitochondrial function and reducing apoptosis in lung epithelial cells subjected to simulated IR conditions (Bojic et al., 2024). Moreover, relaxin-2 has been linked to the attenuation of oxidative stress and the enhancement of post-transplant kidney function, suggesting its potential utility in improving graft viability (Bojic et al., 2024). The appli-

cation of prolactin in islet transplantation has shown to improve β -cell survival, thereby offering a promising adjunct therapy to enhance transplant outcomes (Yamamoto et al., 2010; Ryszka et al., 2016). Finally, the addition of angiotensin IV to preservation solutions has been shown to improve endothelial function and mitigate oxidative stress, underscoring its relevance in enhancing organ preservation methodologies (Tabka et al., 2018). Overall, these findings elucidate the multifaceted roles of hormone drugs in organ preservation, highlighting their potential to improve clinical outcomes in transplantation.

Noble gases

Argon (Ar) and xenon (Xe) have shown promising potential in enhancing organ preservation strategies, particularly in mitigating IR injury during transplantation (Zhao et al., 2014a, 2015a, 2017b). In studies involving liver grafts from DCD pigs, the combination of 0.1 nM dexmedetomidine and 50% Ar significantly reduced various forms of cellular death, including ferroptosis, while preserving hepatocyte integrity and viability (Chen et al., 2024b). Similarly, in a preclinical model of kidney transplantation, Ar-saturated Celsior solution demonstrated superior outcomes compared to standard air-saturated solutions, leading to improved renal function, reduced tubular necrosis, and enhanced survival rates in pigs (Faure et al., 2016). Further research in rat models indicated that both Ar and Xe preserved renal architecture and post-transplant function by lessening IR injury, with Ar showing more pronounced protective effects (Irani et al., 2011; Faure et al., 2016). Xe, in particular, was found to enhance anti-apoptotic markers such as Bcl-2 and heat shock protein 70, providing significant cellular protection during ischaemic insults (Irani et al., 2011). Collectively, these findings suggest that the manipulation of noble gas compositions in preservation solutions offers a novel strategy to enhance graft viability and expand the donor pool, particularly for marginal donors.

Other drugs

Simvastatin has shown great promise in improving liver preservation during transplantation by mitigating IR injury when added to cold storage solutions. Studies demonstrated that flow cessation during cold storage led to endothelial dysfunction due to the downregulation of the vasoprotective transcription factor Kruppel-like factor 2 (KLF2) (Russo et al., 2012). Simvastatin could prevent this decline by maintaining KLF2 expression, thereby reducing liver damage, inflammation, oxidative stress, and endothelial dysfunction in rat livers. Another study explored simvastatin's ability to protect endothelial cells from activation and apoptosis during cold storage by upregulating KLF2 and its target genes, further reinforcing its role in preserving vascular function (Gracia-Sancho et al., 2010). A randomized controlled trial is underway to evaluate the benefits of simvastatin in human liver transplantation, comparing graft survival at 3, 6, and 12 months, which aims to confirm simvastatin's protective effects and its potential to improve long-term liver transplant outcomes (Pagano et al., 2018).

Trophic factors including FR167653 have significantly improved renal recovery and reduced renal fibrosis and inflammation in pig kidney models after cardiac death (Desurmont et al., 2011). TJ-M2010-5, when used with HTK preservation solution, improved cardiac function and reduced apoptosis and inflammatory responses in ischaemic conditions (Yang et al., 2022). Ferrostatin-1 and SUL150 were highlighted for their role in preserving HEK293 cells during cooling, demonstrating potential for enhancing graft survival by preventing ferroptosis and maintaining ATP levels (Gartzke et al., 2023). Alpha-1-antitrypsin was also examined for its anti-inflammatory properties in heart transplantation, targeting IR injury (Korkmaz-Icöz et al., 2023). The ERK signaling pathway inhibitor U0126 and the Sigma-1 receptor agonist fluvoxamine both improved post-transplant kidney function by reducing ischaemic injury markers (Zheng et al., 2021; Hosszu et al., 2023). Notably, H₂S donors like STS and AP39 exhibited the ability to prevent cold IR injury, significantly enhancing graft function and survival in kidney transplantation models (Lobb et al., 2012; Juriasingani et al., 2018). Additionally, liraglutide was shown to improve islet viability and function in type 1 diabetes transplants by reducing oxidative stress (Cai et al., 2024). Other compounds such as AA2G (a vitamin C derivative), taurine, dexmedetomidine, quercetin, and flavonoids have also demonstrated protective benefits in preserving various organs and improving transplantation outcomes (Arata et al., 2004; Chen et al., 2023; Zarnitz et al., 2023, 2024b). Overall, the document highlights the promising potential of these agents in enhancing organ preservation strategies and improving transplant success rates.

Conclusion

This review discusses several mechanisms of regulated cell death associated with organ transplantation. In addition to immune rejection, IR injury is an important challenge in organ transplantation. Mitigating IR injury holds significant potential to increase the usage of DCD donors and shorten the waiting period for transplant recipients. The involvement of various cell death mechanisms in IR injury is complex and varies depending on the specific context. Arguably, organ preservation strategies that intimately target cell death pathways may ultimately improve graft quality and post-transplant outcomes *per se*. Numerous studies have investigated methods of attenuating IR injury by targeting these regulated cell death pathways in different cellular and animal models, which offers promising therapeutic avenues for reducing IR injury in transplantation, potentially improving graft survival and overall transplant outcomes, although the majority strategies are yet in preclinical stage.

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