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Commentary

DUSP12 acts as a novel endogenous protective signal against hepatic ischemia–reperfusion damage by inhibiting ASK1 pathway

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Ischemia–reperfusion injury (IRI) consequent to major liver surgery is a still unmet clinical problem. The activation of endogenous systems of hepatoprotection can prevent the damaging effects of ischemia–reperfusion (IR) as shown by the phenomenon known as 'ischemic preconditioning'. The identification of endogenous signal mediators of hepatoprotection is of main interest since they could be targeted in future therapeutic interventions. Qiu et al. recently reported in *Clin. Sci.* (*Lond.*) (2020) **134**(17), 2279–2294, the discovery of a novel protective molecule against hepatic IR damage: dual-specificity phosphatase 12 (DUSP12). IR significantly decreased DUSP12 expression in liver whereas DUSP12 overexpression in hepatocytes protected IRI and DUSP12 deletion in DUSP12 KO mice exacerbated IRI. The protective effects of DUSP12 depended on apoptosis signal-regulating kinase 1 (ASK1) and acted through the inhibition of the ASK1-dependent kinases c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK). These results enlighten DUSP12 as a novel intermediate negative regulator of the pro-inflammatory and pro-apoptotic ASK1/JNK-p38 MAPK pathway activated during hepatic IR and identify DUSP12 as potential therapeutic target for IRI.

The interruption of blood supply to an organ followed by a subsequent blood reperfusion causes ischemia–reperfusion (IR) injury (IRI) [1]. IRI contributes to morbidity and mortality in a wide range of pathological conditions (i.e. myocardial infarction, ischemic stroke, circulatory arrest) and is a main risk factor in general surgery and organ transplantation. Although IRI represents a general clinical problem, no specific therapeutic options are still available for its cure or prevention [1].

In liver, IRI can induce dysfunction after hepatic resection and is responsible for inducing non-anastomotic biliary strictures and for 10% of early graft failures following transplant [2,3]. Steatosis, the most common hepatic disease in western countries, greatly increases liver susceptibility to IRI and is associated with higher morbidity and mortality upon major liver surgery or transplantation of marginal steatotic liver grafts [4].

IRI is the consequence of several intra- and extra-cellular alterations started by the ischemic period and paradoxically exacerbated at reperfusion [1]. In liver, the lack of oxygen during ischemia causes ATP depletion and impairment of Ca^{2+}, H^+ and Na^+ homeostasis that directly contribute to sublethal or irreversible damage of liver cells [1,5–8]. Notably, ATP loss is furtherly exacerbated in fatty liver where intracellular steatosis increases mitochondrial uncoupling and dysfunction consequent to ischemia [4,15,40]. Upon oxygen re-admission, uncoupled mitochondria of survived cells show a decreased capacity to synthesize ATP and produce augmented amount of reactive oxygen species (ROS) with promotion of oxidative stress. These events augment death or damage of liver cells that release damage-associated molecular patterns (DAMPs) and/or pro-inflammatory chemokines and cytokines giving rise to sterile

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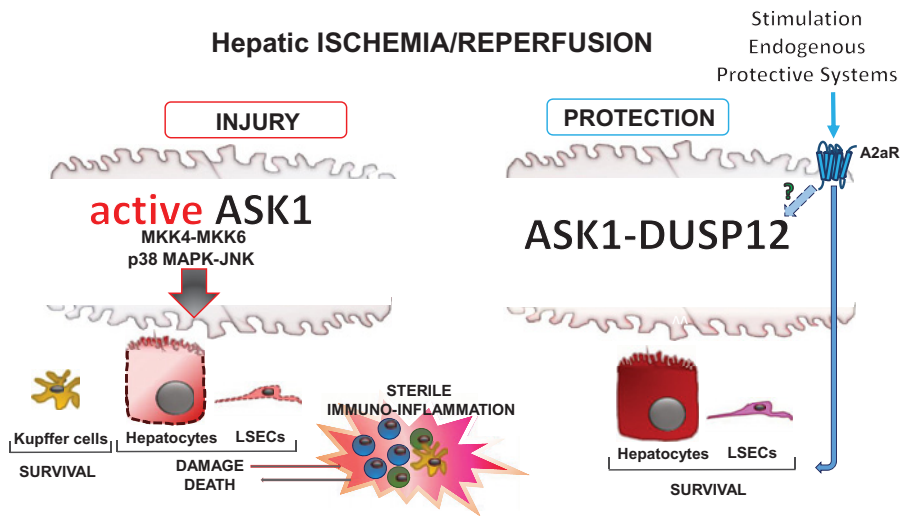

Figure 1. Hepatic IR induces hepatocyte and LSECs damage and death and stimulation of immuno-inflammatory reactions

IRI is induced by a complex pattern of pro-death and pro-inflammatory signals that are mainly and upstream stimulated by active-ASK1 (schemes of involved pathways and mechanisms are in ref: [5–10, 33]). DUSP12 negatively regulates and interacts with ASK1 and is down-regulated by IR. DUSP12 overexpression protects hepatic IRI [17]. Pharmacological preconditioning by activation of A2aR stimulates endogenous systems of tissue protection that also protect IRI (schemes of involved pathways and mechanisms are in ref: [5,33]). As hypothesized in test, hepatic preconditioning might prevent loss or induce the expression of DUSP12, recently identified as novel protective molecule against IRI.

immuno-inflammatory reactions. Resident Kupffer cells and infiltrating neutrophils and lymphocytes produce additional pro-death stimuli, including proteases, FAS ligand (FASL) and tumor necrosis factor (TNF) and high amount of ROS and oxidant species that further increase mitochondrial alterations and induce liver cell death by necrosis, apoptosis, necroapoptosis, mitoapoptosis, ferroapoptosis or autophagy, and subsequent continuous recruitment and activation of inflammatory cells [1,5–8].

The molecular events underlying or possibly counteracting this complex series of damaging conditions are object of intense investigations. Their identification is, in fact, crucial as they represent potential targets for therapeutic interventions against IRI and, specifically, against hepatic IRI. In major liver surgery, pharmacological targeting of critical mediators of hepatic IRI, would be, indeed, of main interest e.g. for extending criteria of donor livers for transplantation or for treating patients with fatty liver undergoing liver resection for hepatocellular carcinoma.

Most of the signal mediators by now implicated in hepatic IRI production are associated with the induction of definite types of accidental or regulated cell death program or of definite immuno-inflammatory processes [5–9]. Molecular factors contextually involved in all or most of the multi-faced damaging IRI processes would be, however, most desirable candidates as therapeutic targets for IRI since their modulation would produce general and more effective outcomes.

In this contest, critically important is the role, recently enlightened by numerous studies, of the Apoptosis Signal-regulating Kinase 1 (ASK1) as master and upstream inductor of IRI in liver (Figure 1) and in other extra-hepatic tissues [10].

ASK1 inhibition or down-regulation has been shown to prevent, in different cell types, necrosis [11], apoptosis [12], mitoapoptosis [13], ferroptosis [14] and autophagy [6] and it has been, also, demonstrated that it is able to abolish hepatocyte death [15–17] and Kupffer cells survival [16] during hepatic IRI. Additionally, ASK1 blockage has proven to protect significantly immuno-inflammatory reactions and parenchymal damage caused by IR in multiple tissues including liver, kidney, nervous and cardiovascular systems [10,15–22].

These observations indicate ASK1 and, alternatively, its molecular effectors or regulators, as suitable targets for IRI therapy.

ASK1 is a member of the large mitogen-activated protein (MAP) kinase kinase kinase (MAP3K) family that activates, downstream two different subgroups of MAP kinase kinases, MAP kinase kinase (MKK)4 and MKK6, the stress-activated kinases: c-Jun N-terminal kinases (JNKs) and p38 MAP kinases [23] (Figure 1).

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JNK and p38 mitogen-activated protein kinase (MAPK) are thus effectors of ASK1 activity, but their stimulation can produce opposite pro-survival or pro-death outcomes in a mode that is largely dependent on the duration of their activation. Short activation is protective and induces repair mechanisms and cell survival, by contrast, sustained activation induces apoptotic and necrotic cell death [10]. Additionally, during hepatic and extrahepatic IRI, cytotoxic and immune-inflammatory processes are associated with a prolonged JNK phosphorylation [6–9,24]. In a clinical contest, thus, direct inhibition of ASK1 effectors, p38 MAPK and JNK, would be problematic because of the dual physiologic and pathological roles of the kinases. Importantly, ASK1 demonstrated to promote the sustained and pro-apoptotic activation of p38 MAPK and JNK [25], but not the short-term p38 MAPK/JNK activation [10]. Thus, direct ASK1 blockage or interventions on mechanisms regulating ASK1 activation could protect IRI abolishing the deleterious effects of the sustained p38 MAPK and JNK stimulation, but not affecting their physiological functions.

The mechanisms inducing ASK1 activation are multiple and include several stress signals such as oxidative stress, endoplasmic reticulum stress, calcium influx, hypoxia/re-oxygenation, DNA damage-inducing agents, lipids load, drugs, infection, radiation and receptor-mediated signaling through TNF or Toll-like receptors [10,16,18–20,26]. ASK1 thus acts as a central sensor of potentially damaging conditions and orchestrate cell response to them. Many of the signals known to activate ASK1 can participate to IRI induction, but others can act independently. This means, ASK1 blockage would induce a detrimental and general inhibition of cells capability to respond to different stresses. Targeting the molecular regulators of ASK1 that are specifically overwhelmed during IRI would be, instead, more appropriate. Up-regulation or maintenance of negative regulators of ASK1 activity in fact, would specifically act against IRI without affecting other stress-responding actions of ASK1.

As generally recognized, ASK1 activity is negatively controlled by redox signaling [10]. In the redox neutral cell, dithiol oxidoreductases, thioredoxin, glutaredoxin and peroxiredoxin bind and maintain ASK1 inactive. Most of the signals responsible for ASK1 stimulation act through an ROS-mediated process. Increased intracellular ROS cause disulfide bonds between the cysteine residues of the dithiol oxidoreductases that dissociate from ASK1 and free ASK1 undergoes activation by auto-phosphorylation [10]. Not all the signals leading to ASK1 activation are, however, ROS-dependent. An important example is ASK1 activation in steatotic or normal liver exposed to I/R. Using steatotic and not steatotic mice hepatocytes exposed to hypoxia/reoxygenation (HR) to mimic '*in vitro*' hepatic IRI, we observed that steatosis further augments ASK1 activation induced by HR [15]. We also found that antioxidants could prevent the increase of ASK1 activation in steatotic hepatocytes but did not affect ASK1 activation induced by HR in not steatotic hepatocytes [15]. These data showed that only lipid-induced ASK1 activation was ROS-dependent whereas ASK1 activation directly induced by HR was unrelated to ROS production [15]. This means that IR can induce ASK1 activation by interfering with ROS-independent mechanisms of ASK1 negative regulation. Such mechanisms, however, are largely unknown.

An important contribution to clarify this issue has been recently published by Qiu et al. [17].

The study of Qiu et al. reports the discovery of a new protective signal against hepatic IRI: the dual-specificity phosphatase 12 (DUSP12). With elegant experiments, Qiu et al. illustrate that overexpression of DUSP12 in hepatocytes prevents hepatic IRI and ASK1 activation [17]. They show that hepatic IRI as well as the stimulation of the ASK1/JNK/p38 MAPK axis, are associated with a decreased expression of DUSP12 [17]. They demonstrate that mice with genetic deletion of DUP12 in hepatocytes present an exacerbated IRI and an increased ASK/JNK/p38 MAPK stimulation. They also report that ASK1 inhibition protects IRI in wt mice but also in mice with DUSP12 overexpressed in hepatocytes [17]. These observations show, for the first time, that DUSP12 depletion during hepatic IRI allows the production of cytotoxic and pro-inflammatory effects dependent on ASK1 activation. They also demonstrate that the capacity of DUSP12 to inhibit JNK/p38 MAPK stimulation during hepatic IRI depends on a negative regulation of DUSP12 on ASK1 activity [17]. This latter observation is consistent with a previous study from the same Authors that reported the protective role of DUSP12 in NASH development and steatosis [27]. This study showed that the protective action of DUSP12 was due to the inhibition of ASK1 activation and demonstrated that such negative regulation relayed on the physical interaction between DUSP12 and ASK1 [27] (Figure 1).

DUSP12 is a member of the dual-specificity phosphatases (DUSPs) family. DUSPs are protein phosphatases that inactivate phospho-threonine and phospo-tyrosine residues of MAPKs by direct interaction. DUSPs regulated expression controls intensity and duration of MAP kinase superfamily (extracellular signal-regulated kinases: ERK, JNK and p38 MAPK) activity to determine the type of their patho-physiological responses [28].

Members of the DUSPs family have been already implicated in IRI prevention. In particular, acute cardiac IRI showed to down-regulate DUSP1 and loss of DUSP1 promoted the activation of JNK [29,30] that, up-regulating the mitochondrial fission factor, induced mitoapoptosis [29]. A more recent report showed that miR-217 protected against myocardial IRI through inactivating NF-κB and MAPK pathways via targeting DUSP14 [31]. Both DUSP1 and DUSP14 thus exert, as well as DUSP12, a protective action against IRI; DUSP1 and DUSP14, however, produce their

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effects by directly inhibiting MAPK activity. By contrast, DUSP12 prevents MAP kinases stimulation, not directly, but through the negative regulation of their upstream inductor, ASK1.

All together, these observations indicate that the 'loss of DUSP12' stands out among other signals involved in IRI, as a process with very suitable properties to become an effective therapeutic target against IRI (Figure 1).

Availability of compounds able to directly target DUSP12 not only for clinical purposes but also for pre-clinical researches is, however, far to be achieved. Drug research for phosphatases, in fact, only recently started overcoming the previous common notion of phosphatases undruggability [41].

An alternative approach to direct DUSP12 targeting, is the identification of conditions able to safely and specifically up-regulate DUSP12 and to make DUSP12 able to exert its protective action against IRI.

Ischemic preconditioning or pharmacological stimulation of one of its main trigger, the adenosine A2a receptor (A2aR), is known to activate a complex endogenous systems of tissue protection that can efficiently protect IRI in liver and in multiple other tissue [5,32–34]. The molecular mechanisms of endogenous hepato-protection involve the stimulation of several signal mediators including, among other, Protein Kinase C (PKC), Protein Kinase A (PKA), Phospholipase C (PLC), Proto-oncogene tyrosine-protein kinase (Src), p38 MAPK and PhosphoInositide 3 kinases (PI3K) [5,33]. This protective pathway increases hepatocytes resistance to hypoxic damage by preventing intracellular pH and Na^+ alterations through the stimulation or expression of constitutive or newly expressed pH regulatory systems such as (Vacuolar-type) V-ATPase [35] or carbonic anhydrase IX [36]. A2aR stimulation also prevents immune-inflammatory reactions and the MKK4/JNK-dependent hepatotoxic and immunolipotoxic effects of lipid load [37,38].

Proteomic analysis of hepatocytes and sinusoidal endothelial cells (LSECs) isolated from control or preconditioned mice liver exposed to IR have shed further light on the pathological processes underlying IR and on their protection by preconditioning [39]. They evidenced changes in several family of proteins mainly involved in cytoprotection, energy metabolism and response to oxidative stress in both control or preconditioned liver exposed to IR [39]. Importantly, peculiar and opposite differences were evident in the pathological (control) and in the protected (preconditioned) liver cells phenotypes [33,39]. The same pathways down-regulated by IR, in fact, resulted up-regulated by preconditioning [39]. This indicates that IR induces a loss of vital enzymes that further decrease the resistance of liver cells to the damaging effects of IR. It also shows that preconditioning is able not only to prevent such loss, but also to increase the cellular content of these same enzymes. Interestingly IRI and steatosis decrease DUSP12 expression in mice liver and such condition allows the cytoxic and pro-inflammatory ASK1/JNK activation [17,27]. Moreover, the novel demonstration of hepatoprotective functions of DUSP12 [17,27], shows that DUSP12 shares the protective properties of the other endogenous cytoprotective signals modulated by ischemic or pharmacological preconditioning. It is thus tempting to investigate whether ischemic or pharmacological preconditioning would antagonize DUSP12 loss during IRI or even promote its increased expression. Such possibility could represent a physiological condition able to overexpress DUSP12, that being an effective protective signal against IRI, would critically contribute to the endogenous protection machinery activated by preconditioning to assure IRI prevention (Figure 1). Unlike direct DUSP12 pharmacological targeting, hepatic preconditioning would have the advantage to be easily inducible during hepatic surgery by intermittent application of the Pringle manouevre [2,5,7,33] or, in general, by the use of pharmacological activators (some of them already approved by FDA for other clinical purposes) of the molecular mediators of hepatic preconditioning [5,33]. A further intervention able to directly [33] and indirectly [42] enhance the hepatoprotective effects of pharmacological preconditioning and, possibly, of DUSP12 over-expression would be the inclusion of antioxidants and of energy-linked metabolites to, i.e., liver graft conservation solution. This condition would exploit the antioxidant and metabolic advantages of preconditioned liver cells augmenting their antioxidant efficiency and their increased capacity to synthesize ATP [39] and thus further counteracting the main pathogenic alterations of IRI: oxidative stress and ATP depletion [1,2,4–7,33].

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

A2aR, adenosine A2a receptor; ASK1, apoptosis signal-regulating kinase 1; DUSP12, dual-specificity phosphatase 12; HR, hypoxia/reoxygenation; IR, ischemia–reperfusion; IRI, IR injury; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein

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kinase; MKK , MAP kinase kinase; NASH, Non alcoholic steatohepatitis; ROS, reactive oxygen species; TNF, tumor necrosis factor.

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