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•Commentary•

MKK4 inhibitor: the hope for liver failure prevention and potential small liver graft transplantation

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Liver regeneration represents a fascinating concept that spans from ancient mythology to modern medical science. In Greek mythology, Prometheus, a hero who defied Zeus by stealing fire and giving it to humanity, was subjected to a severe punishment. He was bound to a rock where, each day, an eagle would feast on his liver, which would then miraculously regenerate overnight. This myth underscores the liver's unique regenerative abilities, a feature that is not just legendary but also scientifically recognized. In contemporary medicine, the liver is known for its remarkable capacity to regenerate after injury. Liver cells typically activate a proliferation program swiftly to repair damage. However, this ability is markedly diminished in cases of acute or chronic liver diseases or when a significant portion of the liver is surgically removed. Such impairments can lead to fatal liver failure. Despite the critical nature of this issue, liver transplantation, the most viable treatment for end-stage liver disease is available to only about 10% of patients, largely due to the scarcity of donor organs. Moreover, there are currently no universally endorsed pharmacological therapies available to enhance liver regeneration, highlighting a significant gap in current medical treatment options.

The research team led by Lars Zender from the University of Tübingen in Germany has been at the forefront of liver regeneration studies for over a decade. In their pivotal 2013 study published in *Cell*, they identified mitogen-activated protein kinase 4 (MKK4) as a key regulator of liver cell regeneration using *in vivo* RNAi screening [1]. This study revealed that the activation of MKK4 by stress signals subsequently activated the JNK1/2/3 and p38 pathways. Further investigations by Zender's team demonstrated that knocking down MKK4 led to the upregulation of the MKK7/JNK1-activated ATF2 and ELK1 transcriptional programs, which were crucial for liver regeneration. Moreover, they observed that livers with silenced MKK4, both in resting and non-regenerating states, showed resistance to Fas-induced apoptosis. Importantly, despite the associations between cancer and increased cell proliferation or resistance to apoptosis, no tumor development was observed in mice with stable intrahepatic knockdown of MKK4. These findings suggest that the absence of MKK4 does not significantly contribute to tumor initiation and establish MKK4 as a promising target for transient pharmacological inhibition.

Human MKK4, also known as MAP2K4, MAPK/ERK-kinase 4 (MEK4), JNK kinase 1 (JNKK1), or SAPK/ERK kinase-1 (SEK1), consists of 399 amino acids [2]. To date, three structures of MKK4 are publicly available. This kinase contains a specific phosphorylation motif, S-X-A-K-T (Ser257 and Thr261), located in the T-loop between subdomains VII and VIII of the kinase domain [3]. Both hydroxy residues are required to be phosphorylated for full activation of the kinase, as mutations of these residues abolish MKK4's activity [4]. Research has established that Ser257 phosphorylation is essential, and Thr261 phosphorylation is required for full MKK4 activation [5]. Recent molecular dynamics (MD) simulations suggest that MKK4, in its inactive and autoinhibited state, likely forms a dimer. This dimer is destabilized by phosphorylation, which in turn activates the kinase [6]. MKK4 shares about 50% of its sequence identity with another kinase, MKK7 [3], which is significant because MKK7 has a

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similar binding pocket. This similarity indicates that MKK7 could interact with small molecule inhibitors in a manner akin to MKK4 [7], making MKK7 a potential off-target when targeting MKK4. Efforts to pharmacologically target MKK4 have included the use of natural products and derivatives, such as 7,3′,4′-trihydroxyisoflavone, genistein, and dehydroglyasperin C. These compounds have been studied to confirm MKK4's viability as a pharmacological target [8-10]. However, despite various reports of MKK4 inhibitors, the targeting precision and effectiveness of these inhibitors remain less than satisfactory.

In their recent preclinical study entitled "First-in-class MKK4 inhibitors enhance liver regeneration and prevent liver failure" published in *Cell* [11], Zender *et al.* introduced a novel MKK4 inhibitor, HRX215. This first-in-class inhibitor has shown promising results in enhancing liver regeneration and preventing liver failure in both murine and porcine models. The implications of their research are profound, potentially ushering in a new era in the management of liver cancer surgery and liver transplantation. Furthermore, HRX215 could significantly improve the treatment protocols for acute and chronic liver diseases.

In their comprehensive study, the authors initially used a doxycycline-induced shMKK4 mouse model, which simulated the systemic effects of MKK4 inhibitors, to explore liver regeneration following partial hepatectomy (PH). Results confirmed that systemic inhibition of MKK4 enhanced liver regeneration. To address potential oncogenic risks, the team also studied fumarylacetoacetate hydrolase (FAH) knockout mice, which are predisposed to spontaneous liver tumorigenesis. Their findings indicated that MKK4 inhibition did not increase the risk of developing liver tumors in these models. Progressing to drug discovery, the authors faced challenges due to the inadequate resolution of available crystal structures of the kinase domain and the absence of a detailed ATP-binding pocket model. Consequently, they selected vemurafenib as the lead compound. After several modifications and employing NMR-based structural analysis, HRX215 emerged as the clinical candidate due to its high selectivity. *In vitro* and *in vivo* evaluations revealed that HRX215 had favorable cell membrane permeability and an elimination half-life of 3.4−3.9 h, supporting its potential for further efficacy and safety analysis. HRX215 promoted hepatocyte proliferation after PH in a dose-dependent manner but did not induce normal liver proliferation, consistent with the effects observed in shMKK4 mice. In addition, the gene expression profiles in livers treated with HRX215 closely mirrored those in shMKK4 mice, underscoring the drug's selectivity. HRX215 also demonstrated protective effects against liver damage, reducing hepatocyte apoptosis in conditions induced by CCl_4 or following PH, and exhibited antisteatotic and antifibrotic effects in mouse models. To evaluate the safety of HRX215, the authors conducted untargeted metabolomics analyses using plasma and liver samples from HRX215-treated mice. These analyses revealed no safety-relevant changes.

To further validate the effects of HRX215 following hepatectomy, the authors employed a porcine model where an 85% liver resection typically triggers acute and lethal post-hepatectomy liver failure (PHLF), a significant clinical challenge. In line with the results from the mouse model, HRX215 significantly increased liver regeneration volume and promoted hepatocyte proliferation in the porcine models. Notably, HRX215 effectively prevented the onset of acute liver failure and maintained normal liver function following the 85% resection. Importantly, HRX215 treatment also prevented postoperative coma and mitigated issues related to reduced liver size and hypoperfusion. These findings indicate that HRX215-mediated liver regeneration was an effective intervention for preventing fatal PHLF after extensive hepatectomy. The authors also conducted a placebo-controlled exploratory phase I first-in-human study on HRX215. This trial, involving 48 healthy male volunteers, did not report any serious adverse events, thereby demonstrating good pharmacokinetics and safety profiles for HRX215.

Overall, this study demonstrated that the first-in-class MKK4 inhibitor HRX215 showed a promising effect in promoting the rapid boost of liver regeneration with excellent safety and pharmacokinetics, which is of great significance (Fig. 1). These findings are crucial as HRX215 offers a much-

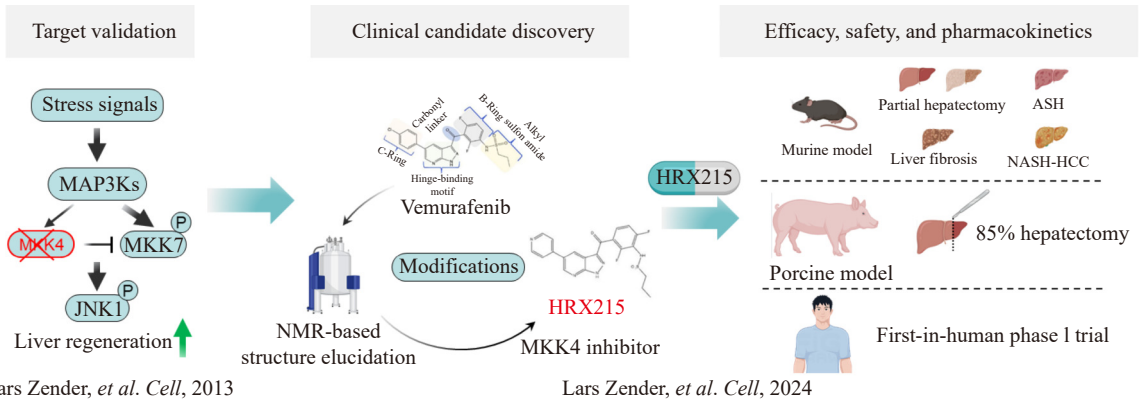

Fig. 1 The discovery of a first-in-class MKK4 inhibitor for liver regeneration. Some elements were modified from FigDraw (www.figdraw.com)

needed alternative for liver tumor resection. Moreover, it addresses the critical issue of organ shortages in liver transplantation, potentially allowing for the use of smaller left liver lobe grafts in normal-sized adults. Importantly, given the role of natural products in the initial target validation and their demonstrated efficacy in inhibiting MKK4, future research should concentrate on identifying more natural compounds with MKK4 inhibitory activity. This exploration should include structural modifications based on structure-activity relationships to develop a broader range of MKK4 inhibitors with high specificity and varied mechanisms of inhibition.

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