The enigma of RIPK1 in the liver: More than just a kinase

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ABSTRACT

Receptor interacting protein kinase 1 (RIPK1) represents a key molecule in cell death. Here, we discuss our recent data on RIPK1 in liver injury and hepatocellular carcinoma development and put these into relation to previous experimental findings to underpin that it exerts opposing kinase-dependent and –independent functions in liver cells.

Hepatocellular carcinoma arises almost exclusively in the setting of chronic liver injury due to various pathogenic factors, triggering a sequence of inflammation, compensatory regeneration and oxidative stress that ultimately drive malignant transformation of hepatocytes.[1] Clinical data and animal models suggest that hepatocyte death is the key trigger of liver disease progression[2], and different modes of cell death such as apoptosis, necrosis, and necroptosis trigger specific cell death responses and promote progression of liver disease through distinct mechanisms.

Receptor interacting protein kinase 1 (RIPK1) is a multifunctional regulator of cell death triggered by tumor necrosis factor and other death ligands.[2] RIPK1 is regulated by posttranscriptional modifications (phosphorylation and ubiquitination) and is part of distinct cell-death related complexes, such as complex IIb (mediating apoptosis) or the necrosome (mediating necroptosis).[2] Based on its central position in cell death regulation, RIPK1 is currently one of the most intensively studied but also most controversially discussed molecules in liver research. Initial evidence for a role of RIPK1 in experimental models of acute liver failure came from studies that relied on usage of a chemical inhibitor of RIPK1's kinase function, necrostatin-1 (Nec-1), suggesting e.g. a role in the mediation of acetaminophen (APAP) toxicity.[3] However, Nec-1 is neither specific for RIPK1, nor is it stable *in vivo* or specific for hepatocytes, and experiments with conditional cre-loxP-based RIPK1 knockout mice in liver parenchymal cells ($RIPK1^{LPC\text{-}KO}$) could not confirm this putative function in the APAP model (**Fig. 1**).[4]

More recently, comparative experimental approaches studying the different functional and biological effects of conditional knockout mice versus mice harboring a kinase-inactive form of RIPK1 ($RIPK1^{K45A}$) could provide important new insights into the opposing functional traits of RIPK1 in liver disease. As such, Filliol and colleagues recently published an interesting study in which they examined the role of RIPK1 in the Concanavalin A (ConA) model of liver injury, which is primarily driven by the activation and recruitment of T cells to

the liver.[5] Using the more specific RIPK1 kinase inhibitor Nec-1s as well as $RIPK1^{K45A}$ kinase-dead mice, the authors saw protection upon inhibition of RIPK1. In contrast, ConA treated $RIPK1^{LPC\text{-}KO}$ mice showed more severe liver damage compared to littermate controls, suggesting that kinase-dependent and -independent functions of RIPK1 differentially regulate the outcome of liver injury.[5]

Mice lacking the IκB-Kinase subunit *Nemo* in LPC ($NEMO^{LPC\text{-}KO}$) fail to activate NF-κB, which drives the spontaneous development of hepatocyte apoptosis, chronic inflammation and liver cancer, a similar sequence as seen in human hepatocarcinogenesis.[6] Recently, Kondylis and colleagues showed that additional ablation of *Ripk1* in $NEMO^{LPC\text{-}KO}$ mice had no significant effect on cell-death activation and hepatocarcinogenesis.[6] In contrast, subcrossing of $NEMO^{LPC\text{-}KO}$ animals with $RIPK1^{D138N}$ kinase-dead mice prevented apoptosis and cancer development.[6] This important finding showed that apoptosis in *Nemo*-deficient hepatocytes is primarily mediated through RIPK1-containing complexes (IIb), and that RIPK1 kinase inactivation keeps inactive Caspase-8 bound to this putative complex. Only when RIPK1 is absent, apoptosis in a *Nemo*-deficient hepatocyte occurs independently of RIPK1, putatively through complex IIa (**Fig. 1**).

We could recently add an additional piece to the puzzle in terms of RIPK1's functions in HCC development. In our study, we examined the phenotype of LPC-specific conditional RIPK1 mice and showed that RIPK1 is dispensable for normal liver homeostasis but withholds an additional TNF-dependent pro-survival function in liver cells.[7] As such, RIPK1 acts as a scaffold to prevent the proteasomal degradation of the E3-ligase TNF receptor-associated factor 2 (TRAF2) in a kinase-independent manner, thereby inhibiting Caspase-8 dependent apoptosis. Interestingly, ablation of *Ripk1* did not affect NF-κB activation in hepatocytes.[7] Moreover, we detected a basal turnover of TRAF2 that was increased in the absence of RIPK1. At present, it remained unclear which specific E3 ligase is targeting TRAF2 for proteasomal degradation and how exactly this process is influenced by RIPK1 in

liver cells. One possibility is that loss of RIPK1 detracts c-IAPs from their proper substrate which is RIPK1 and in turn leads to the conjugation of Ub onto TRAF2, leading to its degradation. Of note, Dupoux and colleagues observed that c-IAP1-mediated TRAF2 degradation is possible in monocytes undergoing macrophage differentiation, but in contrast to our findings in hepatocytes, it did not sensitize those cells to TNF-induced apoptosis.[8] TRAF2 was suggested to support K48-linked ubiquitination and proteasomal degradation of Caspase-8 *in vitro*, thereby tagging Caspase-8 with an "ubiquitin shut-off timer" and curbing apoptosis.[9] This respective scenario is one possible explanation for the observed apoptosis activation in the presence of NF-κB activation in RIPK1$^{LPC\text{-}KO}$ mice.

To confirm the functional relationship between RIPK1 and TRAF2, we finally generated mice with combined ablation of *Ripk1* and *Traf2* in LPC, which led to a dramatic phenotype: RIPK1/TRAF2$^{LPC\text{-}KO}$ mice developed spontaneous LPC apoptosis, which was not only caused by direct activation of Caspase-8 but also defective NF-κB activation, showing that RIPK1 and TRAF2 have redundant functions in NF-κB activation. This spontaneous apoptosis initiated a sequence of regeneration and inflammation that resulted in the spontaneous development of aggressive hepatocellular carcinoma (HCC) development in older mice. Interestingly, histological examination of human HCCs with different etiologies revealed a significant survival benefit for those patients that expressed both, RIPK1 and TRAF2, in their tumors. In contrast, low or undetectable tumor expression of both molecules showed the worst prognosis, which suggested that the new anti-carcinogenic checkpoint controlled by RIPK1 and TRAF2 in mouse livers might be of great relevance in human hepatocarcinogenesis.

Together with the previously published data in various experimental setups, our present data underlined that beyond the previously known functions in NF-κB-, apoptosis-, and necroptosis-activation, RIPK1 harbors an additional, kinase-independent scaffold function that transmits a survival signal via TRAF2 and is important in preventing cancer

(**Fig. 1**). While loss of RIPK1 expression obviously represents a risk factor for cancer development, the present data from several groups and distinct experimental models provide more solid evidence that inhibition of its kinase activity – ideally by more specific and effective new molecular inhibitors – might be a promising strategy not only in the treatment of acute liver injury but also in the chemoprevention of cancer. Moreover, the available experimental data on RIPK1 in liver disease models nicely illustrate the power of combining genetic knockout studies with specific genetic point mutations, not only on a functional level but also in terms of their translation into human disease. Based on the fact that new phosphorylation sites have recently been identified in RIPK1[10], it is more than likely that the story of RIPK1 in liver disease is not over yet but that new genetic tools will reveal more yet unrecognized functions and translational opportunities.

Figure legend

Figure 1. The contribution of RIPK1-kinase-activity and –scaffold-function in the appearance of acute liver injury and HCC-development. Genetic or pharmacological inhibition of RIPK1 kinase activity protects against ConA- and APAP-induced acute liver injury, whereas genetic silencing of RIPK1 is not protective (upper panel). Furthermore, in $NEMO^{LPC\text{-}KO}$ mice, RIPK1 kinase activity mediates HCC development. Under kinase-inactive conditions, RIPK1 scaffold blocks spontaneous hepatocarcinogenesis, whereas loss of RIPK1 again leads to spontaneous hepatocarcinogenesis in this model. Furthermore, RIPK1 scaffold ablation in $RIPK1^{LPC\text{-}KO}$ mice causes TNF-dependent proteasomal degradation of TRAF2 thereby activating apoptosis. The kinase-activity of RIPK1 is dispensable for this process. Consequently, combined ablation of *Ripk1* and *Traf2* in LPC induces spontaneous apoptosis due to impaired NF-κB-activation which enables spontaneous HCC-development (lower panel).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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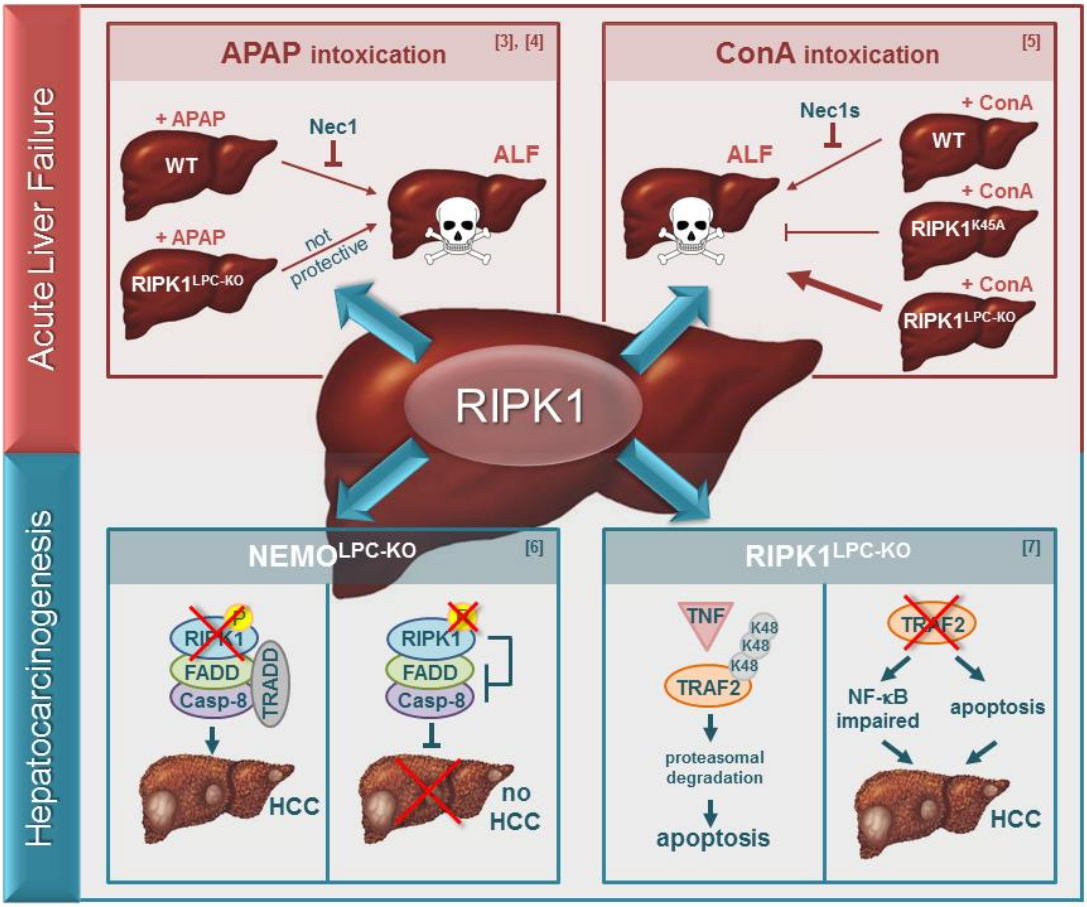
Acute Liver Failure
APAP intoxication
[3], [4]
+ APAP
WT
Nec1
ALF
+ APAP
RIPK1LPC-KO
not protective
ConA intoxication
[5]
Nec1s
ALF
+ ConA
WT
+ ConA
RIPK1K45A
+ ConA
RIPK1LPC-KO
RIPK1
Hepatocarcinogenesis
NEMOLPC-KO
[6]
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FADD
Casp-8
TRADD
HCC
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FADD
Casp-8
no HCC
RIPK1LPC-KO
[7]
TNF
K48
K48
K48
TRAF2
proteasomal degradation
apoptosis
TRAF2
NF-κB impaired
apoptosis
HCC