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Background and Aims: Due to the lack of proper biomarkers and potentially effective treatments, the management of cholangiocarcinoma (CCA) is still challenging. Ion channels have been proven to be novel biomarkers and new targets for cancer therapy, due to their easy druggability. The voltage-gated K+ channel hERG1 exerts pleiotropic effects in cancer cells. This study explored the role of hERG1 in the biology of intrahepatic CCA (iCCA).
Method: Validation of hERG1 in iCCA tissues was performed in TCGA database. In vitro experiments were conducted to estimate the impact of hERG1 inhibition on cell function in iCCA cell lines (HUCCT1, CCLP1, CCA4).
Results: A significant difference in hERG1 gene expression was observed between iCCA and normal tissue samples. Similarly, iCCA cell lines showed significantly higher protein content of hERG1 compared to normal cholangiocytes (NHC3). Treatment with E4031, a selective hERG1 inhibitor, showed a limited impact on cell growth, but a substantial reduction of the invasive capabilities of iCCA cells. Immunoprecipitation assays and immunofluorescence revealed the formation of an active macromolecular complex with $\beta 1$ integrin responsible for VEGF-A activation through AKT signaling. Treatment with a bispecific antibody (scDb: single-chain Diabody) that binds the hERG1-$\beta 1$ complex, negatively impacted the invasiveness of iCCA cells as well as expression of genes regulating epithelial to mesenchymal transition. In vitro co-treatment with scDb and cisplatin-gemcitabine, significantly reduced growth of iCCA cells.
Conclusion: This study indicates that hERG1 may be relevant in promoting the malignant characteristics of iCCA.

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Targeting RuvBL1 reduces mTOR-driven NASH-HCC progression in conditional PTEN-KO mice

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Introduction: RuvBL1 belongs to the highly conserved AAA+ ATPases. It is deregulated in various human cancers and its expression correlates with a worse prognosis in HCC patients. We previously found that RuvBL1 haploinsufficiency impairs the PI3K/Akt/mTOR pathway in liver.
Aim: Given the relevance of mTOR pathway hyperactivation in HCC, we hypothesized that RuvBL1 genetic targeting could reduce mTOR-driven hepatocarcinogenesis.
Material and Methods Results: $Pten^{hep-/-}$ and $Ruvbl1^{hep+/-}$ mice were crossed to generate $Pten^{hep-/-}Ruvbl1^{hep+/-}$ mice. NASH was assessed by histology at 12 weeks of age. Metabolic and inflammatory markers were evaluated by qPCR and IHC. mTOR pathway was analysed by WB of liver lysates. PPARalpha activity was evaluated by luciferase reporter assay. RuvBL1 interactome was evaluated by MS proteomics of RuvBL1-IP. HCC development was assessed by macroscopic tumour count and by histology. AML-12 PTEN KO cells were generated by CRISPR-Cas9 genome editing.

$Pten^{hep-/-}Ruvbl1^{hep+/-}$ developed significantly less steatosis, fibrosis, and inflammation compared to $Pten^{hep-/-}$ mice. The mTOR-driven lipogenic targets were similarly expressed in the two mice models. However, *Ppara* and its target CPT1 was increased in $Pten^{hep-/-}Ruvbl1^{hep+/-}$. The spontaneous and insulin-induced accumulation of lipid droplets in PTEN KO AML-12 cells was completely abrogated by RuvBL1 inhibition with CB-6644. Inhibition of RuvBL1 activity by CB-6644 increased PPARalpha transcriptional activity in AML-12 WT and PTEN KO. Analysis of RuvBL1-IP in AML-12 revealed that RuvBL1 interacts with members of the lysosomal AMPK complex. Furthermore, p-AMPK and p-RAPTOR were increased in $Pten^{hep-/-}Ruvbl1^{hep+/-}$ compared to $Pten^{hep-/-}$ mice. Finally, $Pten^{hep-/-}Ruvbl1^{hep+/-}$ mice aged to 15 months showed better survival than $Pten^{hep-/-}$ which developed significantly more HCC and of higher grade. qPCR analysis showed a significant upregulation of key lipolytic genes, such as *Cpt1a, Acadl, Acadvl* and *Ppara,* in $Pten^{hep-/-}Ruvbl1^{hep+/-}$ at 15 months of age.
Conclusion: RuvBL1 targeting reduces mTOR hyperactivation hampering NASH-HCC progression in $Pten^{hep-/-}$ mice, likely promoting the switch from mTOR-driven lipogenesis to AMPK-induced fatty acid catabolism

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MiR-22 regulates HIF-1A pathway and tumor progression and represents a possible biomarker of sorafenib response in hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) represents the third leading cause of cancer mortality worldwide with approximately 40% of patients diagnosed at advanced stages. Immunotherapy and tyrosine-kinase inhibitors sorafenib and lenvatinib represent the first-line treatments in HCC. Despite immunotherapy has revolutionized HCC management, advanced stages are characterized by limited response and early onset of drug-resistance. The identifica-