EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

A novel player in inflammation and cancer: The deubiquitinase CYLD controls HCC development

Jeremie Gautheron, Tom Luedde*

Department of Internal Medicine III, University Hospital RWTH Aachen, Germany

See Article, pages 995–1003

During the past decades, hepatocellular carcinoma (HCC) became a major health burden and currently represents the third leading cause of cancer-related mortality worldwide [1]. While a continuous decline in cirrhosis-related mortality was observed over the past decades, mortality rates for HCC increased until the end of the last millennium. In fact, the incidence of HCC still nearly equals its mortality rate [2]. The introduction of the multi tyrosine kinase inhibitor sorafenib represented a major breakthrough for the therapy of HCC [3]. However, despite extensive efforts, other chemotherapeutic agents and also alternative tyrosine kinase inhibitors did not show a significant benefit for HCC patients and it is currently unclear which therapeutic strategies beyond sorafenib will be available in the future [4]. It is well established that in most instances HCC arises in a setting of chronic hepatic inflammation, but the knowledge on this unique association between inflammation and cancer has not yet translated into preventive or therapeutic concepts against HCC in patients with chronic liver disease [5], highlighting the need for a better understanding of the function of inflammatory signaling pathways in hepatocarcinogenesis.

Among the cytokines that can induce inflammation, tumour necrosis factor (TNF) withholds a privileged role in the chronically inflamed liver [6]. After its binding to TNF receptors 1 (TNFR1) or 2 (TNFR2), several distinct protein complexes can be assembled, leading to the activation of different signaling pathways, such as the pro -survival and -inflammatory NF-κB pathway, the stress-related Jun-(N)-terminal kinase (JNK) and p38MAPK pathways or the pro-apoptotic caspase cascade [7]. In addition to phosphorylation events, the activation status of these respective signaling pathways is tightly controlled by ubiquitination, which involves the attachment of one or several ubiquitin molecules to a substrate [8]. Until recently, ubiquitination was mainly considered to play a role in controlling the half-life of proteins, but it soon became apparent that ubiquitination also regulates signaling events by acting as modulator of the enzymatic activity or docking of regulatory molecules [8]. To add an additional level of complexity, ubiquitination is a dynamic process that can be counterbalanced by deubiquitinase enzymes, including the molecule CYLD. CYLD was initially discovered in patients with familial cylindromatosis, a rare inherited cancer, characterized by the formation of benign tumours in hairy parts of the body [9]. Later, the group of Gilles Courtois could show that CYLD is an essential modifier of NF-κB and the ubiquitination state of the NF-κB-activating molecule NEMO [10]. In addition to NF-κB, various studies performed mainly in cell culture revealed that CYLD deubiquitinates multiple other members of the TNF-signaling cascade, including TGF-β-activated kinase-1 (TAK1) and the TRAF family of ligases [11] (Fig. 1). Interestingly, expression of CYLD was shown to be dysregulated in several human cancers, including lung and colon cancer and multiple myeloma [12].

In the paper from the group of Henning Schulze-Bergkamen published in the present issue of the *Journal of Hepatology* [13], the authors examined the role of CYLD in liver homeostasis and the development of HCC by applying a conditional, cre/loxP-based knockout approach of CYLD in parenchymal liver cells. Specifically, they did not ablate the whole CYLD protein but only exons 7/8, resulting in overexpression of a naturally occurring, shorter splicing version of CYLD (s-CYLD) that preserves its catalytic activity but lacks the 3rd CAP domain of the protein responsible for binding of e.g., NEMO and TRAF2 [10,14] (CYLDxAlbCre). Expression of this mutant form in CYLDxAlbCre animals resulted in a spontaneous phenotype characterized by prominent biliary fibrosis and ductular reaction, reflecting a chronic inflammatory response with immune cell infiltration and overexpression of inflammatory cytokines and chemokines such as TNF, IL-6, and MCP-1. However, in contrast to other genetic models of chronic hepatic inflammation [15,16], CYLDxAlbCre mice did not develop spontaneous HCCs, but were more susceptible to liver tumours induced by the chemical carcinogen diethylnitrosamine (DEN). Interestingly, some liver tumours in CYLDxAlbCre mice showed histological characteristics of cholangiocellular carcinomas (CCC). On the one hand, this might be explained by the fact that the albumin-Cre line used in this paper is not specific for hepatocytes but mediates deletion in a parenchymal hepatic precursor cell compartment [17]. On the other hand, the detection of CCCs in CYLDxAlbCre mice might support recently published findings by the group of Holger Willenbring showing that CCC can directly

Received 17 July 2012; received in revised form 20 July 2012; accepted 20 July 2012
☆ DOI of original article: 10.1016/j.jhep.2012.06.017.
* Corresponding author. Address: Department of Internal Medicine III, University Hospital RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany.
E-mail address: tluedde@ukaachen.de (T. Luedde).

Editorial

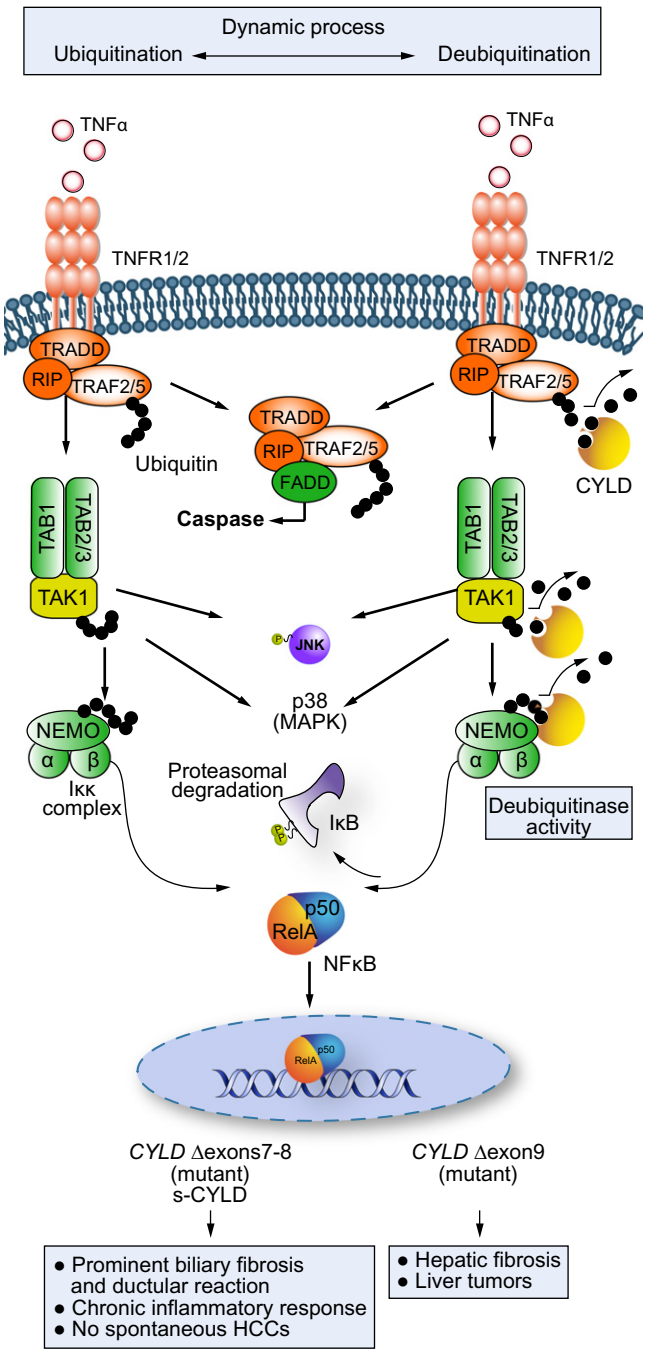

Fig. 1. After binding to its receptor (TNFR1), TNF induces trimerization of the receptor and recruitment of the protein complex I, among others including the adapter protein TRADD, the ubiquitin ligases TRAF2/TRAF5 and the protein kinase RIP1. This complex promotes NF-κB activation through ubiquitination and subsequent recruitment of TAK and the IKK complex. The kinase TAK1 is responsible for the activation of JNK and p38MAPK. CYLD deubiquitinates several molecules of complex I including TRAF2, TAK1 and NEMO, thereby facilitating the formation of complex II, consisting of RIP1, FADD, and procaspase 8, which then initiates apoptosis. Nevertheless, this apoptotic pathway can be inhibited by a sufficient NF-κB stimulation inducing transcription of caspase inhibitors like cFLIP. TNF, tumour necrosis factor; TRADD, tumor necrosis factor receptor type 1-associated death domain; RIP1, receptor-interacting protein 1; TRAF2/5, TNF receptor-associated factor 2/5; FADD, Fas-associated protein with death domain; JNK, Jun-(N)-terminal-kinase; NEMO, NF-κB essential modulator, TAK1, TGF-β-activated kinase-1; p38MAPK, p38 MAP kinase.

evolve from hepatocytes by a pathway involving NOTCH and AKT signalling [18]. Finally, the authors demonstrated that their spontaneous phenotype was associated with a subtle increase in NF-κB signaling reflected by upregulation of NF-κB target genes like *survivin* and *GADD45β*.

The study makes an important contribution to the field of inflammation and hepatocarcinogenesis, since it clearly demonstrates a novel, interesting function of CYLD in HCC development. However, like every good study, it leaves unanswered questions on top of the novel findings presented. While the authors demonstrated increased NF-κB activity in CYLDxAlbCre animals, it is presently not clear if NF-κB is really the main downstream target of CYLD mediating its anti-carcinogenic function, given the multiple other interaction partners of CYLD within the TNF pathway. Of note, it was previously demonstrated that blockage of NF-κB results in increased hepatic tumourigenesis in the DEN-HCC-model [19]. Therefore, it is unclear if the opposite situation in CYLDxAlbCre mice – overactivation of NF-κB – really functionally explains increased tumourigenesis in these animals. In contrast, the phenotype in CYLDxAlbCre animals might be a consequence not of the absence of full-length-CYLD, but of the increased intracellular amount of the short-CYLD-version, putatively lacking binding sites to certain factors like NEMO and TRAF2 in exons 7/8, but still retaining the deubiquitinase-activity encoded from exon 9 on.

Important complementary information to the present study might come from a recent paper published in the journal *Cancer Cell* by the group of Iannis Talianidis [20]. In contrast to the present approach, this group performed a conditional knockout of exon 9 of CYLD, resulting in expression of a deubiquitinase-deficient form of CYLD similar to oncogenic mutations described in humans [21]. Interestingly, expression of this mutant in liver cells resulted in spontaneous development of hepatic fibrosis and liver tumours. On a functional level, certain features of the phenotype seen in that CYLD mutant mouse were mediated through the kinase TAK1, since combined deletions of TAK1 and CYLD led to less fibrosis and periportal cell death than those observed in CYLD single mutants. However, TAK1/CYLD combined mutant animals still showed cancer development and other features of TAK1 single mutant animals [15], demonstrating that neither the absence nor the over-activation of TAK1 is beneficial for liver cells. Nevertheless, given that absence of the deubiquitinase-activity of CYLD results in a much more dramatic phenotype than the exon 7/8 deletion presented in the current paper, it would be of high interest to identify the interaction partners of CYLD that still bind to the short form (s-CYLD) and prevent cell death and spontaneous cancer in CYLDxAlbCre animals. In the light of these two interesting studies and the elegant approach of partial deletion of certain exons in the *Cyld* gene, it is possible that future studies taking similar approaches rather than a complete genetic knockout might lead to novel important functional knowledge of key players in inflammation and cancer, since they are probably closer to mutations observed in human cancer and also more relevant to pharmacological applications.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Sherman M. Hepatocellular carcinoma: epidemiology, surveillance, and diagnosis. Semin Liver Dis 2010;30:3–16.
[2] Cabibbo G, Craxi A. Epidemiology, risk factors and surveillance of hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2010;14:352–355.
[3] de Lope CR, Tremosini S, Forner A, Reig M, Bruix J. Management of HCC. J Hepatol 2012;56:S75–S87.
[4] Villanueva A, Llovet JM. Targeted therapies for hepatocellular carcinoma. Gastroenterology 2011;140:1410–1426.
[5] Vucur M, Roderburg C, Bettermann K, Tacke F, Heikenwalder M, Trautwein C, et al. Mouse models of hepatocarcinogenesis: what can we learn for the prevention of human hepatocellular carcinoma? Oncotarget 2010;1:373–378.
[6] Tacke F, Luedde T, Trautwein C. Inflammatory pathways in liver homeostasis and liver injury. Clin Rev Allergy Immunol 2009;36:4–12.
[7] Luedde T, Schwabe RF. NF-kappaB in the liver-linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2011;8:108–118.
[8] Karin M, Gallagher E. TNFR signaling: ubiquitin-conjugated TRAFfic signals control stop-and-go for MAPK signaling complexes. Immunol Rev 2009;228:225–240.
[9] Bignell GR, Warren W, Seal S, Takahashi M, Rapley E, Barfoot R, et al. Identification of the familial cylindromatosis tumour-suppressor gene. Nat Genet 2000;25:160–165.
[10] Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtois G. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. Nature 2003;424:801–805.
[11] Courtois G. Tumor suppressor CYLD: negative regulation of NF-kappaB signaling and more. Cell Mol Life Sci 2008;65:1123–1132.
[12] Massoumi R. CYLD: a deubiquitination enzyme with multiple roles in cancer. Future Oncol 2011;7:285–297.
[13] Urbanik T, Boger RJ, Longerich T, Becker K, Ehrenberg KR, Hovelmeyer N, et al. Liver specific deletion of CYLDexon7/8 induces severe biliary damage, fibrosis and increases hepatocarcinogenesis in mice. J Hepatol 2012;57:995–1003.
[14] Trompouki E, Hatzivassiliou E, Tsichritzis T, Farmer H, Ashworth A, Mosialos G. CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. Nature 2003;424:793–796.
[15] Bettermann K, Vucur M, Haybaeck J, Koppe C, Janssen J, Heymann F, et al. TAK1 suppresses a NEMO-dependent but NF-kappaB-independent pathway to liver cancer. Cancer Cell 2010;17:481–496.
[16] Luedde T, Beraza N, Kotsikoris V, van Loo G, Nenci A, De Vos R, et al. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. Cancer Cell 2007;11:119–132.
[17] Xu X, Kobayashi S, Qiao W, Li C, Xiao C, Radaeva S, et al. Induction of intrahepatic cholangiocellular carcinoma by liver-specific disruption of Smad4 and Pten in mice. J Clin Invest 2006;116:1843–1852.
[18] Fan B, Malato Y, Calvisi DF, Naqvi S, Razumilava N, Ribback S, et al. Cholangiocarcinomas can originate from hepatocytes in mice. J Clin Invest 2012.
[19] Maeda S, Kamata H, Luo JL, Leffert H, Karin M. IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 2005;121:977–990.
[20] Nikolaou K, Tsagaratou A, Eftychi C, Kollias G, Mosialos G, Talianidis I. Inactivation of the deubiquitinase CYLD in hepatocytes causes apoptosis, inflammation, fibrosis, and cancer. Cancer Cell 2012;21:738–750.
[21] Trompouki E, Tsagaratou A, Kosmidis SK, Dolle P, Qian J, Kontoyiannis DL, et al. Truncation of the catalytic domain of the cylindromatosis tumor suppressor impairs lung maturation. Neoplasia 2009;11:469–476.