Editorial

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Hepatic progenitor cells, senescence and IL-6 as the main players in combined hepatocellular-cholangiocarcinoma development

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Primary liver cancer (PLC) is the fourth leading cause of cancer-related deaths and is having an increasing impact on society (https://gco.iarc.fr/). When thinking of liver malignancies, hepatocellular carcinoma (HCC), the pediatric tumor hepatoblastoma, and cholangiocarcinoma (CCA) first come to mind. However, a neoplasm exhibiting elements of both HCC and CCA within the same nodule had already been reported in 1903, later being called "mixed" or "combined" hepatocellular-cholangiocarcinoma (cHCC-CCA).[1] Although less frequent, the incidence of this malignant entity has been underestimated over the years, mainly due to its difficult histological diagnosis and discrimination from HCC and intrahepatic CCA (iCCA). As such, a significant number of cases have been misclassified as HCC or CCA,[2,3] therefore hindering its molecular characterization and the design of specific cHCC-CCA clinical trials. However, relevant studies are emerging.[3–5] A genomic landscape and a comprehensive comparison of cHCC-CCA with HCC and iCCA[5] revealed that this rare variant constitutes a distinct subtype with different molecular and clinical characteristics. Like the other PLCs, the asymptomatic nature of incipient cHCC-CCA often results in its detection at an advanced stage; its prognosis is worse than that of HCC, but similar to that of iCCA.[6] Still much remains to be known about cHCC-CCA tumors.

Whereas it is mostly accepted that mature hepatocytes and cholangiocytes are the cells of origin of HCC and CCA, respectively,[7] the cell of origin of cHCC-CCA remains a matter of debate. This is an essential issue when it comes to our understanding of the biology and pathogenesis of this rare tumor. Although cHCC-CCA tumors comprise a heterogenous group, their "general" molecular signature of stemness, together with observations in different mouse models, have indeed supported the concept of a stem cell origin.[1] However, other studies suggested the dedifferentiation and subsequent transformation of mature hepatocytes.[6] Moreover, it has recently been agreed that the presence of typical stem-cell features is no longer sufficient to classify a tumor as cHCC-CCA, as this phenotype is also observed in certain subtypes of HCCs and CCAs.[1,6] Therefore, the origin and characteristics of this type of tumor are still poorly understood.

In this issue of *Journal of Hepatology*, Rosenberg *et al.*[6] examine in detail the contribution of hepatic progenitor cells (HPCs) to the development of PLC in the context of chronic inflammation. The study points to this progenitor compartment as the cell of origin of cHCC-CCA, and provides relevant insights into the underlying mechanism.[8] The authors employed the multidrug resistance 2 knockout (*Mdr2*-KO) mouse model that develops progressive liver disease with portal inflammation, sclerosing cholestatic hepatitis and ductular proliferation.[9] It is generally accepted that *Mdr2*-KO mice spontaneously develop HCC by 12-16 months of age.[8–10] However, the authors explored whether at later stages, when the ductular reaction is prolonged, other tumors, in particular cHCC-CCA, could emerge from HPCs in this chronic inflammatory background.

To trace HPCs and their progeny the authors generated a *Mdr2*-KO mouse line in which HPCs were labelled with YFP using the Foxl1 reporter system. When these mice were followed-up for 18 months, half of them developed cHCC-CCA tumors, as indicated by concomitant detection of hepatocytic and cholangiocytic markers. Importantly, these cHCC-CCA tumors arose from HPCs, as shown by the expression of YFP and the progenitor markers CD24 and CD44. These findings were elegantly corroborated in an engineered version of *Mdr2*-KO mice in which HPCs can be specifically ablated in a controlled manner, as these mice showed a significant reduction in the number of cHCC-CCA tumors. At this point, it was important to establish the relevance of the *Mdr2*-KO mouse model to the human disease, and this was achieved by transcriptomic analyses comparing murine and human tumors. These assays not only confirmed the similarity between human and murine cHCC-CCAs, they also clearly differentiated these lesions from HCCs, confirmed the enrichment in stemness-related genes and also established a significant overlap between inflammatory and progenitor pathways in cHCC-CCAs across species.

Most interestingly, these transcriptomic analyses together with immunohistological studies identified the IL-6-gp130-STAT3 pathway, which is known to sustain the stemness niche, as a

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potentially key molecular player in cHCC-CCA tumors. Consistently, functional studies demonstrated that IL-6-neutralizing antibodies indeed reduced cHCC-CCA tumors in *Mrd2*-KO mice, albeit treated mice developed more HCCs. These observations confirm the complex role of IL-6 in hepatocarcinogenesis and prompted the authors to further explore the underlying mechanisms of its involvement.

Single-cell transcriptomic analyses on liver tissues from 18-month-old *Mdr2*-KO mice revealed a higher expression of IL-6 and IL-6R in immune and parenchymal cells, whereas gp130, and the downstream effectors STAT3 and ERK, were found in the HPC population, suggesting a predominant contribution of the IL-6 trans-signaling pathway[11] in HPC proliferation and cHCC-CCA development. Indeed, *in vitro* activation of IL-6 trans-signaling significantly increased HPC proliferation, while its specific blockade reduced the number of cHCC-CCA tumors in mice. This protumorigenic response of HPCs to IL-6 trans-signaling was already known, but IL-6 was specifically produced by tumor-associated macrophages, and as far as it was characterized, only HCC tumors developed.[12] In the case of cHCC-CCA, the authors observed a positive correlation between age, senescence and IL-6 production in the chronically inflamed *Mdr2*-KO livers, thus pointing to senescent cells as the source of IL-6, as part of the senescence-associated secretory phenotype (SASP).

Consistently, *in vivo* ablation of senescent cells with a senolytic agent strongly reduced IL-6 levels and the number of cHCC-CCA tumors.

This and previous studies highlight the complex, and *a priori* contradictory, context-dependent role of IL-6 in liver tumorigenesis. Thus, when acute liver injury occurs, involving hepatocellular loss-of-function and compensatory liver regeneration, a protumorigenic role for IL-6 has been described.[13,14] IL-6 promotes hepatocyte proliferation through the enhancement of DNA synthesis (in cooperation with growth factors)[15] and an increase in genomic instability, defining its protumorigenic character. However, in chronic liver injury associated with high levels of fibrosis and senescence, IL-6, as part of the SASP, has been found to protect against HCC development. In fact, it was demonstrated that the absence of IL-6 signaling leads to a collapse of senescence and the SASP, followed by increased tumorigenesis (HCC) in *Mdr2*-KO mice.[16] Thus, in chronic injury, IL-6 acts to suppress hepatocyte cell division and hepatocarcinogenesis. Strikingly, this same SASP-related IL-6, through the trans-signaling pathway in HPCs, drives cHCC-CCA development.[8] Previous reports showed how IL-6 derived from this inflammatory environment can also promote the retro-differentiation of tumor-derived hepatocytes or cholangiocytes into stem/progenitor cells,[17] which may also further undergo full malignant transformation into cHCC-CCA. Actually, this event represents another hypothesis regarding the identity of the cell of origin of this rare variant of PLC, and constitutes an area of active research.

The study of Rosenberg *et al.* is nevertheless a significant contribution to unravelling the natural history of these combined tumors. It reinforces the hypothesis of the HPC compartment as their cellular origin, and identifies a new role for the complex IL-6 pathway as part of the SASP in the outcome of chronic liver disease (Fig. 1). Moreover, the authors confirm *Mdr2*-KO mice as an excellent model to study HCC development,[8–10] and, most interestingly, identify these mice as a faithful experimental tool to mimic human cHCC-CCA. However, open questions still remain, mainly concerning the human relevance of these findings and their translation to the clinic. What is the specific context in chronic liver disease that defines the role of IL-6 acting in one way or another? Do cHCC-CCA tumors also develop in a senescent microenvironment in humans? If so, are these senescent cells the unique source of IL-6 with protumorigenic trans-signalling effects on HPCs? Would treatment with a senolytic agent be an alternative for patients with cHCC-CCA? A complex role of senescence in liver homeostasis and disease has also been shown to be context-dependent, since targeting senescent cholangiocytes and stellate cells ameliorates fibrosis at early stages,[18] while hepatocyte senescence likely impedes protumorigenic steatohepatitis-associated phenotypes[16] at more advanced phases of the disease. Additional factors and their environmental interconnections should be identified in order to properly understand the dichotomous activity of IL-6 and senescence in liver carcinogenesis. All these considerations make senescence and IL-6 challenging therapeutic targets.

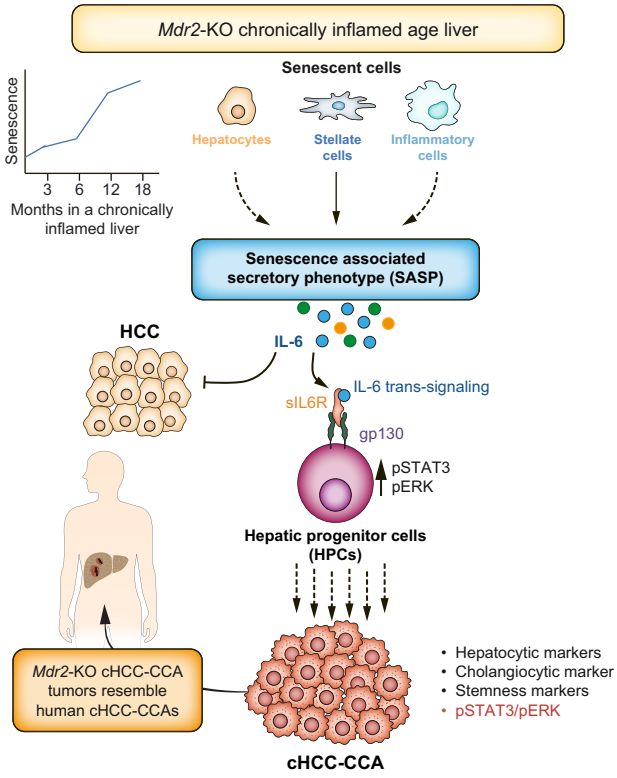

Fig. 1. The *Mdr2*-KO mouse is proposed as an excellent model to study cHCC-CCA tumors. On the background of chronic liver inflammation, senescence contributes to IL-6 secretion as part of the SASP. IL-6 trans-signaling on HPCs promotes their proliferation and cHCC-CCA development. Together with HCC, iCCA and stemness markers, pSTAT3 and pERK activity might contribute to the accurate cHCC-CCA diagnosis. The similarities between the mouse and human cHCC-CCA tumors are corroborated at the level of gene and protein expression, thus postulating the aged *Mdr2*-KO mouse as a faithful experimental tool to mimic human cHCC-CCA. cHCC-CCA, combined hepatocellular-cholangiocarcinoma; HCC, hepatocellular carcinoma; HPCs, hepatic progenitor cells; iCCA, intrahepatic cholangiocarcinoma; SASP, senescence-associated secretory phenotype.

Finally, as mentioned at the beginning, one of the major challenges in cHCC-CCA today is discriminating it from HCC and CCA. In this regard, a very recent work also published in the *Journal of Hepatology* evaluated the largest cohort of patients with cHCC-CCA ever recruited and provided valuable information. In agreement with previous studies,[5,19] this study strengthened data on the stem

cell of origin of these combined tumors and validated the discriminatory potential of the progenitor cell marker nestin to tell cHCC-CCA apart from HCC, although not from iCCA[3]. Taking into account Rosenberg *et al.*'s results, we can speculate that pSTAT3 and pERK activity, together with HCC, iCCA and stemness markers, would help to accurately discriminate cHCC-CCA from HCC and iCCA (Fig. 1). Therefore, Rosenberg *et al.*'s study provides new and relevant data on the cell of origin and the mechanisms implicated in the development of cHCC-CCA tumors as well as a potential signature for their correct clinical identification.

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Conflicts of interest

The authors declare no conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

MA and MGF-B: wrote the manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.09.008.

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Author names in bold designate shared co-first authorship

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