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(Article begins on next page)

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SENP1 activity sustains cancer stem cell in hypoxic HCC

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Hepatocellular carcinoma (HCC) represents the fifth most common cancer and the third leading cause of cancer mortality worldwide, with a minority of patients surviving at 5 years from diagnosis, despite treatment.¹ HCC usually develops in conditions of chronic liver disease (CLD), mostly on the background of a cirrhotic liver, with liver transplantation at present being the only treatment strategy to cure both HCC and the specific CLD. All the other therapeutic strategies, because of the underlying liver cirrhosis, have to take into account, and may be limited in their feasibility, by the residual liver function of the individual patient, a critical parameter affecting the patient's prognosis.² Indeed, even when the surgical intervention is feasible, according to current guidelines, efficient removal of the primary lesions is not often conclusive since intrahepatic recurrence, as well as extrahepatic metastasis, are very frequent and associated with poor prognosis for patients.³ Along these lines, current literature suggests that both the progression of CLD towards HCC development as well as HCC progression and acquisition of resistance to therapy are highly affected by the microenvironment, in which several cells (including tumour associated macrophages or fibroblasts and cancer stem cells (CSCs)), inflammation, fibrosis as well as hypoxia, oxidative stress and autophagy are believed to play a critical role.⁴ In particular, hepatic hypoxia (ie, very common in CLD and HCC) and hypoxia-inducible factors (HIFs) are currently believed to be major determinant players that, in agreement with data in other tumours, can contribute to cancer development and progression by promoting and/or modulating transcriptional programmes (metabolic adaptation, angiogenesis, cell survival, autophagy), inducing epithelial mesenchymal transition and increased invasiveness and/or sustaining or amplifying CSCs compartment.⁵ Indeed, the liver, due to unique morphological and functional organisation (including O₂ gradients in liver zonation), is highly sensible to even modest changes in O₂ partial pressure variation resulting in HIFs-dependent responses. HIFs (particularly HIF-1 and HIF-2, the latter known to be

expressed in hepatocytes and few other cells in the organism) consists of α and β subunits with HIF-1 α and HIF-2 α subunits being oxygen-dependent, whereas the β subunits are constitutively expressed. The α -subunits are carefully controlled under normoxic conditions through mechanisms (hydroxylation on proline or asparagine residues, acetylation of a lysine residue), resulting in either increased proteasome degradation of α -subunits or in the prevention of the formation of the transcriptional complex. In addition, other post-transcriptional modifications, for example, ubiquitination, neddylation, acetylation, phosphorylation, S-nitrosylation and sumoylation, contribute to HIFs stabilisation, protein-protein interaction and HIF functional transcriptional activity, thus modulating the expression of HIFs target genes. Under hypoxic conditions, as in the microenvironment of a growing tumour, control mechanisms decline, HIF α subunits can escape proteolysis, dimerise with HIF-1 β and translocate to the nucleus to promote the expression of a multitude of target genes and non-coding RNAs involved in survival, proliferation, invasiveness, angiogenesis and metabolic adaptation. HIF targets, in turn, will drive tumour progression, metastasis and resistance to chemotherapy⁶ as well as promote the stabilisation/amplification of liver CSCs, which play a role in HCC development and progression,⁷ although the precise identification of CSCs and the mechanisms of their formation remain poorly understood.

In this issue, Cui et al⁸ shed a further light on the correlation between HIF-1 α (and, for some aspects, HIF-2 α as well) and HCC progression, unveiling a new positive feedback loop involving HIF-1 α subunit, overall suggesting the small ubiquitin-like modifier (SUMO) protease 1 (SEN1) as a novel putative therapeutic target for HCC. This message is of relevance since increasing evidence has shown that HIF-1 α is an important SUMO substrate, although HIF-1 α SUMOylation and related effects may vary among different cell types. In their interesting study, the authors first confirmed that hypoxia, through the involvement of both HIF-1 α and HIF-2 α , were able to enhance stemness in HCC cells and also confirmed the previously proposed role of SEN1 in stabilising HIF-1 α under hypoxic conditions via deSUMOylation. More relevant, the authors then demonstrated for the first time that SEN1 is a direct target gene of both HIF-1 α and HIF-2 α , thus providing the first evidence of a positive feedback particularly between HIF-1 α subunit and SEN1. The point is of potential extreme interest because in their study the authors also provide direct evidence that SEN1, a member of the SUMO proteases family including other five members,⁹ is the only member consistently and constantly upregulated in human HCC versus related peritumoural tissue. Moreover, SEN1 expression was found to correlate with a more aggressive tumour behaviour in terms of angiogenesis, metastasis and advanced tumour stage as well as to

CD24 expression and stemness features. Straightforward experiments, involving SENP1 gain and loss of function, then formally demonstrated, in vivo and in vitro, that deSUMOylation is required to stabilise HIF-1 α transcriptional activity preventing its proteasome degradation. Accordingly, specific downregulation of SENP1 (by shSENP1) suppressed in vitro the hypoxia-induced cell stemness and in vivo tumourigenicity in xenograft models, whereas mutation of SUMO sites in HIF-1 α rescued the loss of hypoxia-induced stemness in SENP1-knockdown HCC cells. This means, in practical terms, that the regulatory role of SENP1 on hypoxia-induced enhancement of liver CSC properties is mechanistically dependent on its catalytic activity, making SENP1 a promising putative target for novel therapeutic approaches. Of interest, in their study Cui et al⁸ failed to detect significant conjugation of SUMO with HIF-2 α in HCC cells, contrary to what reported for HeLa cells in which HIF-2 α was found to be regulated by SUMO and SENP1.¹⁰ Accordingly, although SENP1 is upregulated by hypoxia through the involvement of both HIF-1 α and HIF-2 α , only the knockdown of HIF-1 α , but not of HIF-2 α , partially suppressed SENP1-enhanced stemness of HCC cells in hypoxia.

Intriguingly, one has to note that recently Li et al¹¹ demonstrated that the SUMOylation operated by the E3 ligase Cbx4, corecruited with HIF-1 α on vascular endothelial growth factor (VEGF) promoter, can increase the hypoxia induced VEGF expression in HCC. By contrast, Cui et al,⁸ by focusing their study on hypoxia-induced CSC subpopulation, revealed that deSUMOylation is required to promote the transcriptional activity of HIF-1 α on stem cell genes (ie, CD24, Nanog and Oct3/4). Moreover, the authors demonstrated that deSUMOylation is required to promote and/or maintain CD24+ liver CSC population. These interesting data seem to support the emerging hypothesis that post-transcriptional modifications may operate by redirecting the HIFs activity to different pool of target genes.

The overall main message by this study, through the identification of the multiple relationships between hypoxia, HIFs, SUMOylation and HCC stemness is quite clear: SENP1, whose expression is upregulated by both HIF-1 α and HIF-2 α , is an attractive novel target for HCC therapy. This relies on the provided evidence that SENP1, by repressing the SUMOylation of HIF-1 α at K391 and K477 sites by its SUMO protease catalytic activity (ie, activity able to decrease the HIF-1 α stability and transcriptional activity), can upregulate the expression of HIF target genes, including SENP1 and stemness-related genes like Nanog, Oct4 and CD24. This opens a new avenue of therapeutic intervention since the development of efficient and selective inhibitors specifically targeting SENP1, and then HIF-1 α -related transcriptional activity, may result in a significant

interference with HCC growth as well as intrahepatic recurrence and extrahepatic metastasis. This is an urgent need for HCC, a very aggressive tumour with associated high mortality, for which the only validated therapeutic approach relies in the administration of sorafenib, currently recommended as standard treatment for patients with advanced-stage HCC.^{2,12} Moreover, although effective in prolonging the survival of patients with advanced HCC, sorafenib unfortunately induces heavy side effects and multiorgan toxicity, often leading to interruption of treatment in these patients.

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