Editorial

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Hydroxymethylbilane synthase (*aka* porphobilinogen deaminase): A novel metabolic tumor suppressor gene in hepatocellular carcinoma

Antonio Fontanellas[1,2,3,*], Matías A. Avila[1,2,3,*]

[1]*Program of Hepatology, CIMA, University of Navarra, Pamplona, Spain;* [2]*CIBERehd, ISCIII, Madrid, Spain;* [3]*IdiSNA, Pamplona, Spain*

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Hydroxymethylbilane synthase (HMBS), also known as porphobilinogen deaminase (PBGD), catalyzes the third reaction in the process of heme synthesis (Fig. 1A) which takes place in all cells but predominantly in bone marrow erythroblasts and hepatocytes.[1] The first and rate-limiting step in the pathway is the conversion of glycine and succinyl-CoA into δ-aminolevulinic acid (ALA) by the enzyme ALA synthase (ALAS). ALA is then metabolized by ALA deaminase (ALAD) into porphobilinogen (PBG), the substrate of HMBS. Hepatic *ALAS* gene expression, protein stability, cellular localization and enzymatic activity are tightly regulated by heme, the end-product of the pathway. Five more enzymatic steps complete heme synthesis (Fig. 1A). Inactivating mutations in different genes along this pathway define a group of diseases known as porphyrias. Haploinsufficiency affecting *HMBS* is responsible for acute intermittent porphyria (AIP), the most prevalent and severe of the acute hepatic porphyrias (Fig. 1A). These conditions share biochemical and clinical features characterized by attacks of severe neuropathic-type pain associated with a marked overproduction and accumulation of the early intermediates of heme synthesis.[1,2] Buildup of ALA and PBG is particularly exacerbated under conditions of high heme demand, when ALAS1 expression increases and the defective HMBS becomes rate-limiting.

Long-term complications include arterial hypertension, neurological and sensory disorders and chronic renal failure.[1] The development of liver cancer, mostly hepatocellular carcinoma (HCC), is also recognized as a long-term complication of AIP. First evidence came from Scandinavia in the 1980s (reviewed in[3]), and the increased risk of HCC development in patients with AIP was confirmed in a subsequent prospective French study[4] (Fig. 1B). More recently, a meta-analysis revealed a significantly higher prevalence of HCC in female compared to male patients with AIP,[5] which is opposed to the male predominance of HCC in the general population.[6] Interestingly, most patients with AIP that developed HCC did not have cirrhosis, and showed minimal or absent parenchymal inflammation and fibrosis,[3,4,7] which is also in contrast with the high prevalence of cirrhosis-associated HCC in the reference population.[6] These observations suggested the involvement of different mechanisms in AIP-related hepatocarcinogenesis. Notably, earlier reports described a higher frequency of HCC in symptomatic patients with AIP,[8] an observation that was recently corroborated.[9] The severity of AIP symptoms is normally associated with biochemical disease activity, and it is well known that higher disease activity is more common in female patients. This association suggests that AIP-related biochemical alterations, and not female sex, could be a triggering factor for HCC development. As mentioned above, one hallmark of the disease is the accumulation of the heme precursors ALA and PBG (Fig. 1A) in hepatocytes, which is exacerbated during attacks. ALA accumulation can cause oxidative stress, DNA damage and cytotoxicity.[3] Therefore, an adequate metabolic flux of ALA through the heme synthesis pathway would not only preserve heme availability, it would also prevent the development of a potentially carcinogenic microenvironment. In 2015, the detection of an acquired *HMBS* inactivating mutation in HCC tissue from a patient who already carried a germline mutation raised the interesting possibility that *HMBS* could behave as a tumor suppressor gene[10] (Fig. 1B). The notion that metabolic genes could act as tumor suppressors was already put forward about 20 years ago, when, for instance, mice deficient in methionine-adenosyltransferase-1 were found to spontaneously develop HCC,[11] or mutations in tricarboxylic acid cycle (TCA) genes were identified as cancer drivers.[12] Now, Molina and collaborators delved into this issue by analyzing *HMBS* mutations in 4 HCC tissues from 3 patients diagnosed with AIP, and screening 2 large cohorts of sporadic HCC cases (754 total).[13] Remarkably, it was found that all AIP-related HCC samples had acquired a second pathogenic somatic mutation in *HMBS*. On the other hand, *HMBS* mutations were detected in 7 patients of the sporadic HCC series, 5 of whom harbored bi-allelic alterations. Considering the gene size and mutation rate, the authors postulate a positive selection of *HMBS* mutations in HCC. Consistent with the inactivating nature of the identified mutations, PBG was markedly accumulated in tissues from sporadic HCCs with bi-allelic *HMBS* inactivation, and the already elevated concentrations measured in non-tumoral AIP livers were boosted upon second allele inactivation in AIP-HCC tissue. In agreement with the literature, most patients with HCC and mutant *HMBS* were female, devoid of common HCC risk factors and had low fibrosis scores. From a molecular standpoint, activating *CTNNB1* mutations were present in most of them, whereas no *TP53* mutations were found. Transcriptome profiling indicated a homogeneous clustering of these tumors, and their ascription to molecular subclasses associated with Wnt/β-catenin

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* Corresponding authors. Address: Program of Hepatology, CIMA, University of Navarra. Avda. Pio XII, n55. 31008 Pamplona, Spain. Phone: + 34 948 194700.
E-mail addresses: afontanellas@unav.es (A. Fontanellas), maavila@unav.es (M.A. Avila).
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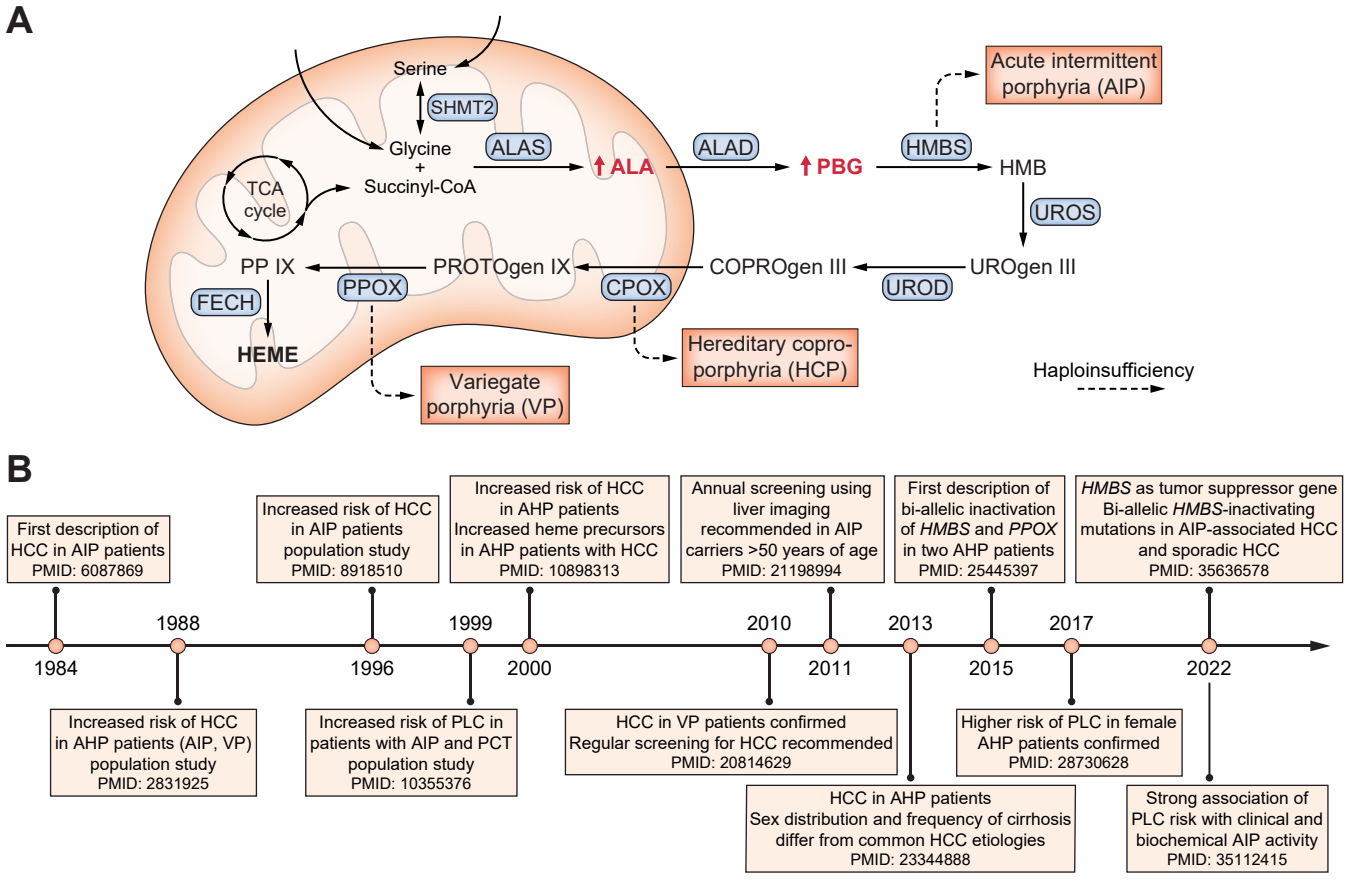

Fig. 1. Heme synthesis pathway and timeline of key developments in our understanding of the link between AIP and HCC. (A) Heme synthesis pathway and associated genetic disorders. AHP include 4 disorders. Haploinsufficiency in the third (HMBS, EC 2.5.1.61), sixth (CPOX, coproporphyrinogen oxidase, EC 1.3.3.3) and seventh (PPOX, protoporphyrinogen oxidase, EC 1.3.3.4) enzymes cause AIP, HCP and VP, respectively. They are characterized by a marked overproduction and accumulation of the early intermediates of heme synthesis (ALA and PBG). Patients with VP and HCP are considered less prone to severe acute attacks and show lower incidence of primary liver cancers than patients with AIP. Homozygous deficiency of ALA dehydratase (ALAD, EC 4.2.1.24) constitutes the fourth type of AHP, a severe ultra-rare disease in which no HCC cases have been described, probably due to the low life expectancy of these patients. (B) Timeline of the main milestones in the identification of the high risk of PLC associated with AIP and the identification of *HMBS* as a tumor suppressor gene. AIP, acute intermittent porphyria; ALA, δ-aminolevulinic acid; FECH, ferrochelatase; HCC, hepatocellular carcinoma; HCP, hereditary coproporphyria; PLC, primary liver cancer; PPIX, protoporphyrin IX; PROTOgen IX, protoporphyrinogen IX; TCA, tricarboxylic acid cycle; SHMT2, serine hydroxymethyltransferase 2; UROD, uroporphyrinogen decarboxylase; UROgen III, uroporphyrinogen III; UROS, uroporphyrinogen III synthase; VP, variegate porphyria.

activation, which are characterized by good differentiation and low proliferative activity.[14] According to their DNA methylation profile, *HMBS*-inactivated HCCs also resembled sporadic *CTNNB1*-mutated tumors characterized by profound DNA methylation alterations, as recently described by this same research group.[15]

The critical biological role of the heme group elicits the intriguing issue of how tumor cells with bi-allelic *HMBS* inactivation would thrive under limited availability of this cofactor. In fact, *HMBS* gene expression is significantly induced in HCCs in association with reduced patient survival, as observed in the TCGA HCC cohort (http://ualcan.path.uab.edu). The residual HMBS activity of mutant enzymes, and/or heme uptake from the extracellular milieu, could compensate for the lack of its biosynthesis. Nevertheless, in patients with germline *HMBS* mutations, overall heme availability is limited; therefore, this issue warrants further investigation. Regarding the tumorigenic processes triggered by bi-allelic *HMBS* mutation, accumulation of toxic levels of heme precursors such as ALA, and to a certain extent PBG, can certainly be considered. However, cytochrome deficiency due to reduced heme availability can also lead to enhanced mitochondrial production of reactive oxygen species, as well as to impaired reactive oxygen species scavenging by heme-dependent antioxidant enzymes.[16] Nevertheless, the observations presented by Molina *et al.* also suggest the potential involvement of additional mechanisms. As could be expected, *HMBS*-deficient tumors showed a remarkable upregulation of *ALAS1* expression and accumulated significant amounts of PBG. This implies a remarkable consumption of the ALA precursors glycine and succinyl-CoA[17] (Fig. 1A). Consistently, these tumors displayed increased expression of genes of the serine-glycine biosynthetic pathway such as *PSAT* and *SHMT2*. Importantly, besides driving glucose towards glycine synthesis, activation of this side-branch of the glycolytic cascade significantly contributes to DNA synthesis and proliferation in tumor cells.[18] Intriguingly, SHMT2 was recently shown to interact with and stabilize β-catenin, promoting the growth of colorectal cancer cells.[19] This novel non-metabolic function of SHMT2 may define an additional crosstalk with the Wnt/β-catenin pathway in *HMBS*-deficient tumors, even in the absence of *CTNNB1* mutations.

Heme biosynthesis is closely linked to the TCA cycle, from which it derives succinyl-CoA.[17] This cataplerotic reaction is

likely taking place in *HMBS*-deficient HCCs, as suggested by the compensatory upregulation of citrate synthase observed in these tumors.[13] Among the consequences of exacerbated succinyl-CoA consumption can be a depletion in the levels of its precursor α-ketoglutarate (α-KG). α-KG is not only an intermediary metabolite in the TCA, it is a critical cofactor for DNA and histone demethylases, core epigenetic regulators whose enzymatic activities are significantly affected by intracellular α-KG fluctuations.[20] Interestingly, and as discussed by the authors, Wnt/β-catenin activation as well as *CTNNB1* mutations also enhance the expression of heme pathway genes, including *ALAS1* and *ALAD*. Therefore, *HMBS* deficiency and *CTNNB1* mutations may concomitantly impact on the levels of α-KG, and consequently on the activity of epigenetic regulators. Accumulation of epigenetic alterations may be relevant to the hepatocarcinogenic process slowly unfolding in the context of *HMBS* mutation, and thus are worth being further explored.

The study by Molina and collaborators supports the notion that *HMBS* is a *bona fide* tumor suppressor gene in HCC. As carriers of a germline mutation, patients with AIP would be at an increased risk of bi-allelic *HMBS* inactivation by developing a second somatic mutation, which would foster malignant transformation. The authors also identified bi-allelic somatic *HMBS* alterations in tumors from patients without any known HCC risk factors. Remarkably, among these cases there were 2 patients carrying AIP-causing pathogenic germline mutations. As the prevalence of likely-pathogenic *HMBS* mutations in the Caucasian population is ~1:1,700,[7] the involvement of *HMBS* deficiency in HCC development in non-cirrhotic livers may be underreported in asymptomatic carriers. This report indeed improves our understanding of hepatocarcinogenic mechanisms and reveals new avenues for future research. It also further emphasizes the importance of devising safe and effective therapies for patients with AIP and active disease who are certainly at increased risk of developing HCC.[2]

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Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions

Both authors made equal contribution.

Supplementary data

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Author names in bold designate shared co-first authorship

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