SETDB1 is a new promising target in HCC therapy

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Hepatocellular carcinoma (HCC), a major form of adult primary liver cancer, is the third cause of cancer-related deaths worldwide. The development of this tumor has been associated with various risk factors, mostly chronic viral hepatitis, chronic alcohol consumption and aflatoxin-B contaminated food, and shows higher incidence in cirrhotic patients. Current therapy is as yet very limited, thus American and European guidelines recommend implementation of surveillance programs in high risk patients. Liver transplant and interventional radiology are still the most efficient treatments while chemoembolization represents the choice for unresectable HCC. The sole drug currently showing some survival benefits in HCC patients in advanced stages and preserved liver function is sorafenib, an inhibitor of tyrosine kinases affecting proliferation and angiogenesis (1). Novel pharmacological approaches against HCC are strongly needed.

Along with various genetic causes, recent findings described HCC onset and progression deeply correlated to epigenetic modifications [reviewed by (2)] of both DNA and histones (i.e., methylation and hydroxymethylation of DNA cytosine residues and acetylation, ribosylation, phosphorylation, ubiquitination, sumoylation, methylation, deamination and proline isomerization of histone tails). These local modifications control gene specific transcriptional activity and often, in HCC as well as other tumor types, aberrant patterns of epigenetic marks cause the silencing of tumor suppressor genes (3). Notably, epigenetic modifications are reversible, thus representing attractive targets in therapeutic approaches. For these reasons, each enzyme that bears activity of chromatin modifier and appears correlated to tumor onset and progression holds promise for therapeutic targeting in cancer treatment. Of note, some drugs against epigenetic modifiers have already been used in clinical trials with interesting results. For example, the histone deacetylase inhibitors belinostat and, more recently, resminostat have been assessed in the treatment of HCC patients and have shown signs of efficacy (4,5). In particular, resminostat treatment resulted in a control rate of the disease close to 90% in patients with confirmed progression on prior sorafenib treatment (5).

Notably, Wong and colleagues (6) correlated the upregulation of the methyltransferase SETDB1 with HCC progression, aggressiveness and poor prognosis; moreover they also provided evidence regarding the functional role of SETDB1 in tumoral cell proliferation and invasiveness.

SETDB1 [SET domain, bifurcated 1/ESET/KMT1E (7)] catalyzes the methylation of lysine 9 of histone H3 (H3K9), a well-conserved mark for transcriptional silencing [also catalyzed by other methyltransferases (HMTs), including suppressor of variegation 3-9 homolog 1 (SUV39H1) and SUV39H2 (8), G9a (9), Riz1/PRDM2 (10), CLLD8/KMT1F (11)].

In particular SETDB1, that methylates lysine 9 up to trimethylation (H3K9me3), is responsible for the silencing of heterochromatin (12,13) and euchromatin sequences (7) and has a critical role in early embryonic development. Among the genes, those identified as targets of SETDB1-

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mediated repression are tumor suppressors RASSF1A and P53BP2 (14). As a parallel activity, SETDB1 acts as protein methylase and, interestingly, a recent report pointed to a role in HCC for this enzyme in the methylation of the tumor suppressor p53 (15).

Wong and colleagues started their study by whole-transcriptome sequencing (RNA-seq) comparing the expression levels of a large set of the known chromatin modifiers in HBV-associated primary HCC to correspondent NT livers. Overall, several epigenetics regulators were found modulated, highlighting the relevance of epigenetic mechanisms in controlling aberrant HCC gene expression. In particular, SETDB1 was found significantly upregulated at both RNA and protein levels; this result was confirmed in several *in vitro* models of HCC. Notably, SETDB1 expression levels were also associated to clinicopathological features and survival rates of patients and this analysis suggested its overexpression to have a prometastatic role and a correlation to poor prognosis. To investigate the possible direct functional role of SETDB1 in HCC, these authors knocked-down the expression of this enzyme in two cell models (Hep3B and MHCC97L) and tested the effects of its silencing; SETDB1 knockdown was found to be able to suppress proliferation *in vitro* and reduce tumor size in *in vivo* orthotopic livers. Moreover, coherently with the finding of an up-regulation of SETDB1 in patients' metastases, its knock-down was found to attenuate HCC lung metastasis in orthotopic implants in nude mice. RNA-seq was then used to investigate the global transcriptional modulation in SETDB1 knock-down cells and, as expected, the enriched target genes identified by these analyses belong to multiple pathways often deregulated in cancer, including those involved in the control of cell-cell adhesion. Data were further validated by ChIP and RT-qPCR analysis.

This study also focused on mechanisms of SETDB1 upregulation in HCC and, of note, multiple levels of control have been identified. Firstly, the *SETDB1* gene copy gain was found at chromosome 1q21 (this chromosomal region is frequently amplified in human HCC). Secondly, aiming at the identification of putative consensus binding site for transcriptional regulators on *SETDB1* gene they performed an in silico analysis that allowed for identification of the specificity protein 1 transcription factor (SP1); its role as transcriptional activator of *SETDB1* gene was further confirmed by luciferase reporter assay and inactivation by mithramycin A treatment or siRNA silencing. Finally, the post-transcriptional regulation of SETDB1 was found to be mediated by the down-regulation of the microRNA-29.

Overall these data are of interest not only for the clarification of the oncogenic role of SETDB1 in HCC development but also because these results integrate with other recent findings contributing to the identification of SETDB1 as a new relevant marker of HCC. In fact, recently Fei and colleagues (15) identified in the SETDB1-mediated di-methylation of the tumor suppressor p53 a mechanism by which this methyltransferase executes its role in HCC.

Moreover, SETDB1 has been found to interact with the DNA methyltransferase DNMT3A (14). Interestingly, miR-29 family members are also known to target DNMT3s in HCC cells (16): low levels of miR-29 and DNMT3A modulation have been correlated to HCC aggressiveness (17) and the treatment with a DNMTs inhibitor is able to impair metastasis (18). On the other hand, miRs-29 levels are controlled to maintain the differentiated hepatocyte phenotype (19). Thus, miR-29 family members control methylation activity on both histones and DNA.

In our view, while more efforts are needed to better clarify the role of SETDB1 in all HCC stages, particularly with respect to specific cancer-related targets, the provided evidence indicates this enzyme as a promising target in future therapy. Therefore, further development of specific inhibitors suitable to clinical use is opportune.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Pascual S, Herrera I, Irurzun J. New advances in hepatocellular carcinoma. World J Hepatol 2016;8:421-38.
2. Amicone L, Citarella F, Cicchini C. Epigenetic regulation in hepatocellular carcinoma requires long noncoding RNAs. Biomed Res Int 2015;2015:473942.
3. Calvisi DF, Ladu S, Gorden A, et al. Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. J Clin Invest 2007;117:2713-22.

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4. Yeo W, Chung HC, Chan SL, et al. Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase I/II study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Research Group. J Clin Oncol 2012;30:3361-7.
5. Bitzer M, Horger M, Giannini EG, et al. Resminostat in combination with sorafenib as second-line therapy of advanced hepatocellular carcinoma - The SHELTER Study. J Hepatol 2016;65:280-8.
6. Wong CM, Wei L, Law CT, et al. Up-regulation of histone methyltransferase SETDB1 by multiple mechanisms in hepatocellular carcinoma promotes cancer metastasis. Hepatology 2016;63:474-87.
7. Schultz DC, Ayyanathan K, Negorev D, et al. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev 2002;16:919-32.
8. O'Carroll D, Scherthan H, Peters AH, et al. Isolation and characterization of Suv39h2, a second histone H3 methyltransferase gene that displays testis-specific expression. Mol Cell Biol 2000;20:9423-33.
9. Tachibana M, Matsumura Y, Fukuda M, et al. G9a/GLP complexes independently mediate H3K9 and DNA methylation to silence transcription. EMBO J 2008;27:2681-90.
10. Steele-Perkins G, Fang W, Yang XH, et al. Tumor formation and inactivation of RIZ1, an Rb-binding member of a nuclear protein-methyltransferase superfamily. Genes Dev 2001;15:2250-62.
11. Falandry C, Fourel G, Galy V, et al. CLLD8/KMT1F is a lysine methyltransferase that is important for chromosome segregation. J Biol Chem 2010;285:20234-41.
12. Rowe HM, Jakobsson J, Mesnard D, et al. KAP1 controls endogenous retroviruses in embryonic stem cells. Nature 2010;463:237-40.
13. Sarraf SA, Stancheva I. Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. Mol Cell 2004;15:595-605.
14. Li H, Rauch T, Chen ZX, et al. The histone methyltransferase SETDB1 and the DNA methyltransferase DNMT3A interact directly and localize to promoters silenced in cancer cells. J Biol Chem 2006;281:19489-500.
15. Fei Q, Shang K, Zhang J, et al. Histone methyltransferase SETDB1 regulates liver cancer cell growth through methylation of p53. Nat Commun 2015;6:8651.
16. Kogure T, Kondo Y, Kakazu E, et al. Involvement of miRNA-29a in epigenetic regulation of transforming growth factor-β-induced epithelial-mesenchymal transition in hepatocellular carcinoma. Hepatol Res 2014;44:907-19.
17. Parpart S, Roessler S, Dong F, et al. Modulation of miR-29 expression by α-fetoprotein is linked to the hepatocellular carcinoma epigenome. Hepatology 2014;60:872-83.
18. Ding W, Dang H, You H, et al. miR-200b restoration and DNA methyltransferase inhibitor block lung metastasis of mesenchymal-phenotype hepatocellular carcinoma. Oncogenesis 2012;1:e15.
19. Cicchini C, de Nonno V, Battistelli C, et al. Epigenetic control of EMT/MET dynamics: HNF4α impacts DNMT3s through miRs-29. Biochim Biophys Acta 2015;1849:919-29.

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