Editorial

Deciphering ZIC2/OCT4 signaling as a vulnerability in liver cancer stem cells

Stephanie Ma

School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Correspondence to: Dr. Stephanie Ma. School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China. Email: stefma@hku.hk.

Comment on: Zhu P, Wang Y, He L, *et al*. ZIC2-dependent OCT4 activation drives self-renewal of human liver cancer stem cells. J Clin Invest 2015;125:3795-808.

Submitted Sep 22, 2016. Accepted for publication Oct 09, 2016

doi: 10.21037/tcr.2016.10.85

View this article at: http://dx.doi.org/10.21037/tcr.2016.10.85

Hepatocellular carcinoma (HCC) is the fifth most commonly diagnosed and the second most lethal cancer type, causing an estimated 700,000 deaths in the world, annually (1). In the United States, the incidence of HCC has doubled over the past two decades. The prognosis of the disease is dismal, with only 30% of patients eligible for curative treatments, including liver transplantation or surgical resection, owing to late diagnosis and the presence of underlying liver disease. Although chemotherapy and molecular targeted therapy (sorafenib) is often administered to patients with inoperable HCC as an adjuvant or palliative regimen, tumor recurrence rate is high, partly attributable to the presence of residual self-renewing cancer stems cells (CSCs) that survive initial treatment (2). Identifying novel diagnostic/prognostic markers and therapeutic targets for HCC, in particular those that aim at the CSC subpopulation, is much needed for managing HCC and improving the long-term survival of patients. A paper by Zhu *et al*. (3) recently published in *The Journal of Clinical Investigation*, has provided new insight in this aspect, highlighting the importance of ZIC2, OCT4 and the nuclear remodeling factor (NURF) complex in maintenance of stemness in human liver CSCs. In fact, the research team led by Fan *et al*. has in the past 2 years published a number of key articles where they identified a number of critical signaling pathways that contributes to sustain liver CSC self-renewal, including the interplays of lncTCF7 and Wnt (4), C8orf4, N2ICD and Notch2 (5), as well as lnc-β-Catm and EZH2-dependent β-catenin stabilization (6).

Master transcription factor-mediated transcriptional regulation is one of the key regulatory mechanisms to determine cell-fate changes and is thus of immense value to investigate how they contribute to cancer stemness. By transcriptome microarray analysis of liver CSCs marked by $CD13^{+}CD133^{+}$ surface signature and non-liver CSC counterparts ($CD13^{-}CD133^{-}$) isolated from Huh7 and Hep3B cells, Zhu *et al*. identified 10 transcription factors (TFs) that were highly expressed in liver CSCs (E2F7, OCT4, TCF4, BATF, HSF5, PHTF2, TFAP2A, HELT, ZIC2 and MYPOP). By systematic functional knockdown studies using *in vitro* sphere formation as a readout, they found ZIC2 depletion to result in the strongest inhibitory effect on oncosphere formation and thus focused their study on this zinc finger TF. ZIC2 overexpression in liver CSCs isolated from both HCC cell lines and clinical samples was subsequently validated and high ZIC2 expression in HCC was also confirmed by analysis in a number of large online/publicly available-cohort datasets. By CRISPR/Cas9 knockout or lentiviral-based overexpression approaches, ZIC2 was found to be functionally required for the maintenance of liver CSC self-renewal and tumor propagation. Mechanistically, ZIC2 initiated activation of OCT4 signaling via binding to its promoter and thereby enhancing both the chromatin accessibility and the H3K4 tri-methylation level of the OCT4 locus. This was elegantly demonstrated by ChIP, luciferase reporter, gel EMSA and DNase I digestion assays, ZIC2/OCT4 expression correlation studies as well as ZIC2/OCT4 rescue experiments. More interestingly, the authors then found ZIC2 to recruit the NURF complex, composed of RBBP4, BPTF and SNF2L, to the OCT4 promoter, in a chromatin-binding fashion; and that this recruitment is required for

self-renewal of liver CSCs. Clinically, similar to ZIC2, expression levels of BPTF, a core component of the NURF complex, in HCC also closely correlated with clinical severity and worst overall prognosis.

This paper by Zhu *et al*. is exciting and remains to date the only publication reporting the clinical and functional importance of ZIC2 and/or NURF complex in HCC, liver CSCs and CSCs of any tumor type, as well as the only study to bridge ZIC2/NURF complex to OCT4 activation. OCT4, being one of the four Yamanaka factors (7), is a well-known core transcription factor that plays a role in establishing and maintaining the pluripotency of stem cells. OCT4 has also been shown in the past to be highly overexpressed in liver CSCs and HCC, highlighting its important role in hepatocarcinogenesis, but how it is regulated remains poorly understood. A study by Song *et al*. have found ATOH8, a member of the bHLH gene family, to transcriptionally repress OCT4 and other stem cell-associated genes including NANOG and CD133 in HCC, suggesting that OCT4 can be controlled at multiple layers to modulate cancer stemness (8). Downstream of the molecule, a recent study by our own group have unraveled the involvement of Oct4 in regulating miR-1246 expression in $CD133^+$ liver CSCs, to cooperatively drive Wnt/β-catenin (9). However, whether ZIC2/NURF complex and miR-1246/Wnt can really be linked by OCT4 to contribute to HCC stemness remains to be determined.

Liver CSCs have been functionally identified and linked to various cell surface phenotypes including EpCAM (10), CD133 (11,12), CD24 (13), CD13 (14), CD90 (15), CD47 (16), aldehyde dehydrogenase (ALDH) (17) and CD44 (18). Our understanding of hepatic CSC biology is growing rapidly with a number of critical molecules identified that converges to several common signaling pathways including Wnt/β-catenin, TGF-β, AKT, ERK, STAT3, JNK, more or less regardless of the surface markers used to identify the subpopulation, suggesting that many of these deregulated pathways are commonly shared by these subsets. It will be interesting to see if ZIC2/NURF complex is similarly up-regulated in other liver CSC subpopulations marked by EpCAM, CD24, CD90, CD47, ALDH and CD44 and whether targeting them would lead to depletion of CSCs. The study by Zhu *et al*. have focused on self-renewal as a readout using *in vitro* spheroid formation and *in vivo* limiting dilution and serial transplantation as functional assays. Whether ZIC-dependent OCT4 activation will drive other cancer and stemness properties like metastasis or resistance to therapy would also be worthwhile to follow up, in particular since a previous study by Wang *et al*. have delineated a role of Oct4 in mediating chemotherapeutic drug resistance in liver cancer cells through the AKT/ABCG2 pathway (19). As mentioned by Zhu *et al*. in the discussion section of the paper, ZIC2 was found to be highly expressed in 2/3 partial hepatectomy-regenerated liver cells, suggesting that ZIC2 plays a crucial role in the maintenance and survival of not only liver CSCs, but also normal liver stem/progenitor cells. Of note, OCT4 (20) and CD133 (11) have also been reported to be similarly upregulated during liver regeneration. A better understanding of how OCT4 and ZIC2 are regulated in a normal and cancer setting would also shed light on how these targets can be better used as a future therapy for HCC, sparing normal liver cells along the way. In addition, investigation of whether the NURF complex can be recruited on the c-MYC promoter for its activation in liver CSCs and HCC would also be of interest, given that studies have previously shown this link in pancreatic cancer (21) and also in regulating self-renewal of hematopoietic stem cells (22) and that c-MYC is also commonly overexpressed in HCC and liver CSCs (23).

In summary, through a combination of online-cohort datasets analyses, *in vitro* and *in vivo* functional studies in cell lines and clinical samples and molecular/biochemical approaches, Zhu *et al*. have elegantly demonstrated the novel role of ZIC2 in activating OCT4 to drive self-renewal of human liver CSCs, which can potentially be used as targets for eradicating liver CSCS for future therapy.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Bo Zhai (Department of Hepatobiliary Surgery, The Fourth Hospital of Harbin Medical University, Harbin, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.10.85). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved.

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Cite this article as: Ma S. Deciphering ZIC2/OCT4 signaling as a vulnerability in liver cancer stem cells. Transl Cancer Res 2016;5(Suppl 4):S722-S724. doi: 10.21037/tcr.2016.10.85