



A transcriptional-microRNA network for β -catenin-driven stemness in hepatocellular carcinoma

William K. K. Wu^{1,2,3}, Matthew T. V. Chan¹, Alfred S. L. Cheng^{2,3,4}

¹Department of Anaesthesia and Intensive Care, ²State Key Laboratory of Digestive Diseases, The Chinese University of Hong Kong, Hong Kong SAR, China; ³Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China; ⁴School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

Correspondence to: William K. K. Wu, PhD, FRCPATH. Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Hong Kong SAR, China. Email: wukakei@cuhk.edu.hk; Alfred S.L. Cheng, PhD. School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China. Email: alfredcheng@cuhk.edu.hk.

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Introduction

Wnt/ β -catenin pathway is important in maintaining stemness of embryonic, adult and cancer stem cells (CSCs) in various organs including liver. However, the mechanisms underlying its deregulation in liver CSCs are poorly understood. Ma and colleagues have recently demonstrated that Oct4/microRNA-1246 signaling axis activates Wnt/ β -catenin signaling in a subset of liver CSCs, providing a mechanistic basis for future diagnostic, prognostic and therapeutic developments.

The cancer stem cells (CSCs) are posited to be responsible not only for tumor initiation but also for tumor relapse and metastasis due to their capacities of self-renewal, drug resistance, and phenotypic reversibility (1). Since the Wnt/ β -catenin signaling plays key roles in both adult liver progenitors and CSCs for the regulation of liver embryogenesis, regeneration, and carcinogenesis, detailed characterization of its regulatory mechanisms in CSCs is therefore imperative for improving early detection, prevention and treatment approaches for hepatocellular carcinoma (HCC) (2,3). In this regard, a recent study by Ma and colleagues (4) has revealed a transcriptional-microRNA (miRNA)- β -catenin axis that can induce “stemness” of HCC for self-renewal, tumorigenicity, metastasis and chemoresistance.

Diverse regulation of Wnt/ β -catenin signaling in liver cancer

Around half of all HCC patients have activation of the Wnt/ β -catenin pathway as a result of gene mutations, epigenetic modifications, or other means. While the frequencies of *CTNNB1* and *AXIN* gene mutations are relatively low (5–20%) in human HCCs, accumulating evidence has underscored the importance of epigenetic deregulation of Wnt/ β -catenin signaling via DNA methylation and histone modifications (5). For examples, histone modifiers such as enhancer zeste homolog 2 (EZH2) and histone deacetylase 8 (HDAC8) are frequently over-expressed in human HCCs and contribute to constitutive β -catenin activation via epigenetic silencing of Wnt antagonists (*AXIN2*, *NKD1*, *PPP2R2B* and *PRICKLE1*) (6,7). Recent studies also unveiled a cell cycle-related kinase (CCRK)/GSK-3 β kinase cascade in promoting β -catenin-driven hepatocarcinogenesis (8-10). It is therefore conceivable to anticipate multifaceted regulation of Wnt/ β -catenin signaling in liver CSCs.

Transcriptional-miRNA control of Wnt/ β -catenin in liver cancer stemness

Through miRNA profiling in a CD133⁺ liver CSC subset with β -catenin activation, Ma and colleagues identified

a human miRNA, miR-1246 that specifically suppresses the expression of *AXIN2* and *GSK-3 β* , two members of the β -catenin destruction complex, leading to nuclear accumulation and activation of β -catenin (4). Using a constitutively active β -catenin construct ($\Delta 45\beta$ -cat) that is resistant to phosphorylation-mediated ubiquitination, the investigators demonstrated that β -catenin activation mediates miR-1246-induced tumorigenicity, metastasis and stemness of CD133⁺ liver CSCs. Clinically, endogenous and secretory miR-1246 over-expression in HCC clinical samples was found to be tightly associated with poor patient survival rates, suggesting its potential application as diagnostic and prognostic biomarker for HCC. Through siRNA-mediated knockdown coupled with chromatin immunoprecipitation and gene expression analysis, another important self-renewal molecule overexpressed in CD133⁺ liver CSCs, Oct4, was shown to directly up-regulate miR-1246 expression in HCC cells, thus highlighting an Oct4/miR-1246 signaling axis that drives Wnt/ β -catenin activation in HCC. These data nicely uncover a new layer of non-genetic mechanism by which the Wnt-mediated stem cell-like properties of HCC cells are induced and sustained, providing important insights for future diagnostic, prognostic and therapeutic developments. Perhaps a missing link that warrants investigation is whether Oct4 could induce Wnt/ β -catenin activation by miR-1246, the result of which might further strengthen the functional significance of this miRNA in connecting these two crucial self-renewal pathways (11). Besides, a recent study has shown that the transcription factor ZIC2 is highly expressed in CD133⁺CD133⁺ liver CSC subset and initiates Oct4 activation (12), thus suggesting a transcriptional-miRNA cascade for β -catenin-driven stemness in HCC.

MiRNA-mediated regulation of cancer stemness in HCC

MiRNAs are an important class of posttranscriptional regulators and have been demonstrated to be involved in a variety of cellular processes, such as cell proliferation, differentiation and apoptosis. Emerging evidence supports that miRNAs actively participate in the regulation of stemness in human cancer of different tissue origins (13). The deregulation of miRNAs in liver CSCs was first reported by Ji *et al.* in 2009. Through microarray-based miRNA profiling, the authors demonstrated that miR-181 family members were overexpressed in epithelial cell

adhesion molecule (EpCAM)⁺/ α -fetoprotein (AFP)⁺ liver cancer cells that exhibited stem cell features, including self-renewal and ability to form aggressive tumors *in vivo*. Importantly, inhibition of miR-181 reduced the number of EpCAM⁺ HCC cells and tumor-initiating ability, whereas exogenous miR-181 exerted opposite effects. Mechanistically, miR-181 was found to target CDX2 and GATA6 (two important hepatic transcriptional regulators of differentiation) as well as NLK (an inhibitor of Wnt/ β -catenin signaling) (14). Using a similar approach, Ma *et al.* demonstrated that overexpression of miR-130b promoted stemness in CD133⁺ liver CSCs through targeting TP53INP1 (a positive regulator of p53 signaling) (15). Since then, the number of stemness-regulating miRNAs identified in HCC has continued to grow (Table 1) (16-32). Interestingly, many of the identified miRNAs were found to regulate cancer stemness via direct or indirect modulation of Wnt/ β -catenin signaling as illustrated in Figure 1. The current paper by Ma and colleagues further discovered that miR-1246 connects Oct4 and Wnt/ β -catenin signaling in the regulation of cancer stemness in HCC (4).

Future perspectives

As master control of gene expression, miRNAs themselves exhibit differential expression in liver CSCs. These deregulated miRNAs modulate stemness of HCC cells through extensive interaction with intracellular signaling pathways, including the Wnt/ β -catenin signaling. Further delineation of the upstream and downstream mechanisms of miRNA deregulation with the state-of-the-art sequencing technologies, such as single-cell epigenomics (33) and transcriptomics (34), will help us better understand CSC biology in HCC and discover novel molecular targets for the development of stem cell-specific therapeutics. In particular, it is tempting to investigate if the distinct miRNA expression profile could give rise to synthetic lethality in liver CSCs. To this end, inhibiting upregulated miRNAs in a systematic manner will help elucidate if targeting a particular miRNA can exclusively induce cell death in HCC stem cells while sparing normal cells. As potential non-invasive biomarkers, it will be interesting to determine if circulating miRNAs could serve as surrogates for tissue stem cell markers for stratifying HCC patients with different clinical outcomes. With these in mind, it is hopeful that stemness-related miRNAs will achieve clinical utilities in the management of HCC in the near future.

Table 1 MicroRNAs over- or under-expressed in liver cancer stem cells and their molecular targets

MicroRNAs	Deregulation	Stem cell population	Upstream mechanism	Functional effects upon overexpression	Expression of stem cell markers upon overexpression	Experimentally verified targets	References
miR-21	Upregulated	SP cells		Promote migration and invasion		PTEN, RECK and PDCD4	(16)
miR-92b	Downregulated	EpcAM ⁺		Promote proliferation and inhibit differentiation		CEBPB	(17)
miR-122	Downregulated	CD133 ⁺		Inhibit spheroid formation		PDK4	(18)
miR-130b	Upregulated	CD133 ⁺		Promote chemoresistance, tumorigenicity and self-renewal		TP53INP1	(15)
miR-142-3p	Downregulated	CD133 ⁺		Inhibit self-renewal, tumorigenicity, migration, invasion, angiogenesis and chemoresistance		CD133	(19)
miR-148a	Downregulated	HCC samples with cancer stem cell-like signature		Inhibit proliferation, EMT, migration, invasion and tumorigenicity	Reduced CD44 and CD90	ACVR1, WNT1	(20,21)
miR-150	Downregulated	CD133 ⁺		Inhibit spheroid formation and tumorigenicity; Promote cell cycle arrest and apoptosis	Reduced CD133	c-Myb	(22)
miR-155	Upregulated	EpcAM ⁺ AFP ⁺		Promote spheroid formation, colony formation, migration and invasion	Enhanced EpcAM	CEBPB	(23)
miR-181	Upregulated	EpcAM ⁺ AFP ⁺		Promote spheroid formation and tumorigenicity	Enhanced EpcAM	CDX2, GATA6 and NLK	(14)
miR-192-5p	Upregulated	SP cells		Promote proliferation and metastasis		SEMA3A	(24)
miR-200a	Downregulated	SP cells		Inhibit spheroid formation, EMT, migration, invasion, tumorigenicity	Enhanced EpcAM, CD133, AFP, ABCG2 and CK19	CTNNB1	(25,26)
miR-214	Downregulated	Recurrent HCC samples		Inhibit invasion, colony formation and tumorigenicity	Reduced EpcAM	CTNNB1 and EZH2	(27)
miR-216a/miR-217	Upregulated	Recurrent HCC samples	TGF- β	Promote spheroid formation, migration, and metastatic ability	Enhanced EpcAM	PTEN and SMAD7	(28,29)
miR-429	Upregulated	EpcAM ⁺	Promoter hypomethylation	Promote self-renewal, proliferation, chemoresistance and tumorigenicity	Enhanced Oct4	RBBP4	(30)
miR-452	Upregulated	Chemoresistant hepatospheres		Promote spheroid formation, migration, invasion and tumorigenicity		Sox7	(31)
miR-589-5p	Downregulated	CD90 ⁺		Inhibit spheroid formation, migration and invasion	Reduced Oct4, Sox2 and Nanog	MAP3K8	(32)
miR-1246	Upregulated	CD133 ⁺	Oct-4	Promote self-renewal, drug resistance, tumorigenicity and metastasis		AXIN2 and GSK3 β	(4)

Only deregulated microRNAs with functional effects on stem-cell like phenotypes are shown. AFP, α -fetoprotein; EpcAM, epithelial cell adhesion molecule; SP, side population; TGF- β , transforming growth factor- β .

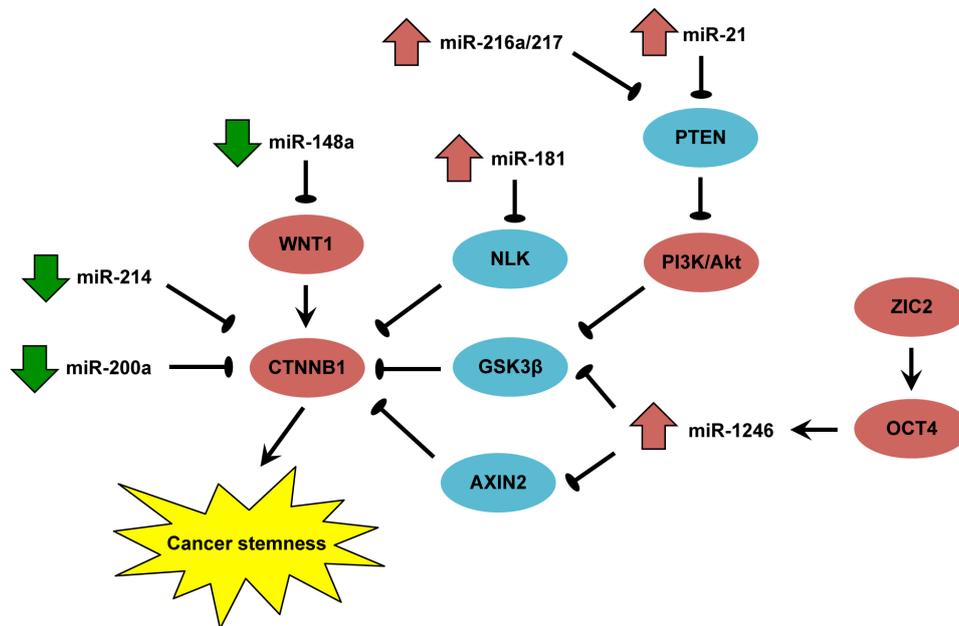


Figure 1 MicroRNA-mediated regulation of cancer stemness in HCC via Wnt/ β -catenin signaling.

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Footnote

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Ethical Statement: The authors are 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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