Perspective

Clinical implications of microRNAs in liver cancer stem cells

Stella Chai[1] and Stephanie Ma[1,2]

Abstract

The prognosis of patients diagnosed with hepatocellular carcinoma (HCC) is often dismal, mainly due to late presentation, high recurrence rate, and frequent resistance to chemotherapy and radiotherapy. Accumulating evidence on the differential microRNA (miRNA) expression patterns between non-tumor and HCC tissues or between liver cancer stem cells (CSCs) and non-CSC subsets and the significant clinical implications of these differences suggest that miRNAs are a promising, non-invasive marker for the prognosis and diagnosis of the disease. This perspective article summarizes the current knowledge of miRNAs in liver CSCs and highlights the need for further investigations of the role of miRNAs in regulating liver CSC subsets for possible future clinical applications.

Key words MicroRNA, liver cancer stem cells, hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the most common form of liver cancer, representing 80% of all cases[1]. The disease is the sixth most common and third most deadly cancer worldwide[2]. The prognosis of patients diagnosed with HCC is often dismal, mainly due to the late presentation, high recurrence rate, and frequent resistance to chemotherapy and radiotherapy[3]. Currently available serum biomarkers, such as alpha-fetoprotein (AFP) and des-gamma carboxy prothrombin (DCP), are insufficient. Thus, there is an urgent need to identify additional sensitive and specific biomarkers, especially markers that can also serve as possible therapeutic targets for the disease[4].

In the past decade, the concept of the cancer stem cell (CSC) or tumor-initiating cell (T-IC) has been vigorously investigated, with encouraging results that suggest a new direction in cancer treatment. In contrast to the abundant non-tumorigenic cancer cells, CSCs comprise a small subset of cancer cells within a tumor that exhibit both stem cell-like and cancer cell-like characteristics, including the abilities to self-renew and to initiate tumor formation[5-7]. There is now evidence to show that CSCs represent a subset of cells that are more resistant to conventional therapy and thus serve as the origin of the entire tumor. Therapies that specifically target these cells, which are believed to be pivotal for the growth and maintenance of the entire tumor mass, may result in a more durable response and even in a cure for the disease. Liver CSCs were first identified as a side population (SP) in 2006[8] and subsequently further characterized to express specific cell surface markers including CD133[9-11], CD90[12], CD44[13], EpCAM (CD326)[14], and CD24[15]. Researchers have also investigated the molecular pathways that regulate this specific group of cells, and several signaling pathways related to cell proliferation and cell differentiation have been largely explored, including Wnt/β-catenin, transforming growth factor-beta (TGF-β), Hedgehog, Notch, and Myc[16].

The discovery of microRNAs (miRNAs) nearly two decades ago has shed much light on the development of cancer research. miRNAs are small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. The first identified miRNAs, *lin-4* and *let-7*, were found to regulate stem cells in *C. elegans*[17,18]. In addition to regulating stem cells, miRNAs play a role in CSC self-renewal, differentiation, tumorigenesis, drug resistance, and metastasis. To date, over 1,000 human miRNAs have been identified, and hundreds of these molecules have been reported to be associated with cancerous tissues and/or body fluids or waste products from cancer patients[19]. As a result, miRNAs have been proposed to function as useful diagnostic and/or prognostic tumor markers in various cancer types[20]. Approximately 100 miRNAs have been found to be deregulated in HCC patients compared with healthy individuals[21] (**Table 1**). In addition, due to the stability of

Authors' Affiliations: [1]Department of Clinical Oncology, [2]State Key Laboratory for Liver Research, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, P. R. China.

Corresponding Author: Stephanie Ma, Department of Clinical Oncology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Room 53, 10/F, Laboratory Block, 21 Sassoon Road, Pok Fu Lam, Hong Kong, P. R. China. Tel: +852-2819-9785; Fax: +852-2816-9129; Email: stefma@hku.hk.

doi: 10.5732/cjc.013.10038

Table 1. Summary of microRNA (miRNA) deregulation in liver CSCs, HCC tissue, and HCC serum/plasma

miRNA	Sample source	Regulation	Target	Reference(s)
let-7	Oct4+CD133+ liver CSCs	Up	–	[28]
miR-130b	CD133+ liver CSCs	Up	TP53INP1	[29]
miR-145	–	–	Oct4	[32]
miR-150	CD133+ liver CSCs	Up	c-Myb	[31]
miR-181	EpCAM+AFP+ liver CSCs	Up	CDX2, GATA6, NLK	[25]
miR-199a-3p	–	–	CD44	[33]
let-7c	Serum	Up	–	[28]
let-7f	Serum	Up	–	[28]
miR-1	Serum	Up	–	[28]
miR-15b	Serum	Up	–	[30]
miR-16	Serum	Down	–	[41]
miR-17-5p#	Serum	Up	–	[42,43]
miR-18a#	Serum	Up	–	[44]
miR-21#	Serum/plasma	Up	–	[27,41,45,46]
*miR-21**	Serum	Down	–	[47]
miR-25	Serum	Up	–	[28]
miR-92a	Serum	Up	–	[28]
miR-130b	Serum	Up	–	[30]
miR-122#	Serum	Up	–	[27,47]
*miR-146a**	Serum	Up	–	[48]
miR-183	Serum	Up	–	[30]
miR-199a-5p#	Serum	Down	–	[41]
miR-206	Serum	Up	–	[28]
miR-215	Serum	Up	–	[48]
miR-221#	Serum	Up	–	[46]
miR-222#	Serum	Up	–	[46,47]
*miR-223**	Serum	Up	–	[27,47]
miR-224#	Serum	Up	–	[46,48]
*miR-375**	Serum	Up	–	[28]
miR-574-3p	Serum	Up	–	[48]
miR-885-5p	Serum	Up	–	[48]
miR-17-5p	Tissue	Up	–	[43]
miR-18a	Tissue	Up	ESR1	[49]
miR-19a	Tissue	Down	–	[50]
miR-21	Tissue	Up	PTEN, PDCD4	[51–53]
miR-22	Tissue	Down	HDAC4	[54]
miR-23a	Tissue	Up	PGC-1α, G6PC	[55]
miR-24	Tissue	Down	–	[50]
miR-26a	Tissue	Down	IL-6	[56]
miR-29	Tissue	Down	Bcl-2, Mcl-1	[57]
miR-29c	Tissue	Down	TNFAIP3	[58]
miR-30d	Tissue	Up	GNAI2	[59]
miR-31	Tissue	Up	–	[51]
miR-34a	Tissue	Down	c-met	[60]
miR-99a	Tissue	Down	IGF-1R, mTOR, PLK1	[61,62]
miR-100	Tissue	Down	PLK1	[62]
miR-101	Tissue	Down	SOX9, Mcl-1	[63,64]
miR-106b	Tissue	Up	APC	[65]
miR-122	Tissue	Up	–	[51]
miR-124	Tissue	Down	ROCK2, EZH2	[63,66]
miR-125a-5p	Tissue	Down	MMP11, VEGF-A, SIRT7	[67,68]
miR-125b	Tissue	Down	SIRT7	[68]

(*To be continued*)

Table 1. Summary of microRNA (miRNA) deregulation in liver CSCs, HCC tissue, and HCC serum/plasma (*continued*)

miRNA	Sample source	Regulation	Target	Reference(s)
miR-126	Tissue	Down	–	[50]
miR-129-3p	Tissue	Down	SOX4	[69]
miR-138	Tissue	Down	CCND3	[70]
miR-139	Tissue	Down	ROCK2	[71]
miR-140-5p	Tissue	Down	TGFBR1, FGF9	[72]
miR-143	Tissue	Up	FNDC3B	[73]
miR-145	Tissue	Down	IRS1, IRS2, IGF1R	[51,74]
miR-146a	Tissue	Down	–	[51]
miR-147	Tissue	Up	–	[50]
miR-151	Tissue	Up	RhoGDIA	[75]
miR-155	Tissue	Up	–	[76]
miR-182	Tissue	Up	MTSS1	[77]
miR-183	Tissue	Up	PDCD4	[78]
miR-191	Tissue	Up	TIMP3	[79]
miR-193b	Tissue	Down	CCND1, ETS1	[80]
miR-195	Tissue	Down	Cyclin D1, CDK6, E2F3	[81]
miR-198	Tissue	Down	c-met	[82]
miR-199a-3p	Tissue	Down	mTOR, c-met, CD44	[33,83]
miR-199a-5p	Tissue	Down	DDR1	[83,84]
miR-200c	Tissue	Down	–	[51]
miR-210	Tissue	Up	VMP1	[85]
miR-214	Tissue	Down	HDGF, β-catenin	[86,87]
miR-216a	Tissue	Up	TSLC1	[88]
miR-219-5p	Tissue	Down	GPC3	[89]
miR-221	Tissue	Up	–	[51]
miR-222	Tissue	Up	–	[51]
miR-223	Tissue	Down	–	[50,51]
miR-224	Tissue	Up	CDC42, CDH1, PAK2, Bcl-2	[90]
miR-301a	Tissue	Up	Gax	[91]
miR-335	Tissue	Down	–	[92]
miR-338-3p	Tissue	Down	Cyclin D1	[93,94]
miR-372	Tissue	Up	–	[95]
miR-373	Tissue	Up	PPP6C	[96]
miR-375	Tissue	Down	AEG–1	[97]
miR-450a	Tissue	Down	DNMT3a	[98]
miR-490-3p	Tissue	Up	ERGIC3	[99]
miR-519d	Tissue	Up	CDKN1A/p21, PTEN, AKT3, TIMP2	[100]
miR-520b	Tissue	Down	MEKK2, Cyclin D1	[101]
miR-520e	Tissue	Down	NIK	[102]
miR-550a	Tissue	Up	CPEB4	[103]
miR-602	Tissue	Up	RASSF1A	[104]
miR-615-5p	Tissue	Up	IGF–II	[105]
miR-637	Tissue	Down	LIF	[106]
miR-650	Tissue	Up	ING4	[107]
miR-657	Tissue	Up	TLE1	[108]
miR-886-5p	Tissue	Down	–	[50]

CSCs, cancer stem cells; HCC, hepatocellular carcinoma. #miRNAs reported to have the same regulation (direction of change in expression) in both serum and tissue samples from HCC patients. *miRNAs reported to have the opposite regulation (direction of change in expression) in serum and tissue samples from HCC patients, –, information not available.

miRNAs in the blood and the tissue-specific characteristics of the miRNA expression pattern, the detection of circulating miRNA levels in the serum of HCC patients is now increasingly recognized to be a promising non-invasive tool for the prognosis and diagnosis of this disease[22]. Recently, an increasing number of studies have found altered miRNA expression in liver CSC subsets compared with non-CSC subsets or normal liver tissue (**Table 1**); other studies have identified various miRNAs that control the expression of liver CSC markers[4,23,24]. Of these, *miR-181, let-7, miR-130b, miR-150, miR-145,* and *miR-199a-3p* are described in detail below. Taken together, these findings suggest that miRNAs may represent tools for the more accurate prognosis and diagnosis of HCC and may even act as potential therapeutic targets for the eradication of liver CSC subsets.

miR-181

Ji *et al*. [25] have found that multiple members of the *miR-181* family, including *miR-181a, miR181b, miR181c,* and *miR181d,* are consistently up-regulated in the liver CSC subset marked with EpCAM+AFP+ surface markers. In this study, the expression of *miR-181* transcripts enhanced the quantity and tumorigenicity of EpCAM+ HCC cells by regulating the expression of the proliferation markers CDX2 (caudal type homeobox 2), UGT2B7 (UDP glucuronosyltransferase 2 family, polypeptide B7), and CYP3A4 (cytochrome P450, family 3, subfamily A, polypeptide 4) and the beta-catenin-related genes CCND1 (cyclin D1) and TACSTD1 (epithelial cell adhesion molecule, EpCAM). Further, *miR-181s* maintained stemness by directly targeting GATA6 (GATA-binding protein 6) and CDX2 (caudal type homeobox 2) to block cell differentiation and NLK (nemo-like kinase) to activate the Wnt/β-catenin pathway. In addition, the expression of *miR-181* transcripts was directly induced upon activation of the Wnt/β-catenin pathway and was inhibited upon its inactivation. This study defined a novel regulatory link between *miR-181s* and human EpCAM+ liver CSCs and implied that the molecular targeting of *miR-181* may be used to eradicate HCC[25,26]. Meng *et al*.[23] have identified the preferential expression of *miR-181* family members in an Oct4+CD133+ liver CSC subset and subsequently identified a critical role for *miR-181s* in Twist-driven metastasis in this specific subset of CSCs. The group further found *miR-181* to directly target RASSF1A, TIMP3, and NLK in Oct4+CD133+ liver CSCs. Despite the consistent preferential expression of different *miR-181* family members in various liver CSC subsets (EpCAM+AFP+ or Oct4+CD133+), the combined expression levels of *miR-181a* and *miR-181c* were not significantly different in serum collected from HCC patients and chronic HBV carriers or healthy individuals[27].

let-7

In addition to *miR-181*, the results reported by Meng *et al*.[23] showed that *let-7* was preferentially expressed in the Oct4+CD133+ liver CSC subset compared to HCC cells, HepG2 cells, or normal liver stem cells isolated from adult human liver tissue. The inhibition of *let-7* increased the chemosensitivity of liver CSCs to sorafenib and doxorubicin, concomitant with an enhanced expression of two downstream targets, caspase-3 and SOCS1[23]. They have also found IL-6, which is commonly overexpressed in HCC, to directly enhance the expression of *let-7a* and *let-7b*. *let-7c* and *let-7f* have been reported to be highly expressed in the serum samples of hepatitis B virus (HBV) carriers and HBV-positive HCC patients, respectively[28].

miR-130b

In our previous study, we have reported the preferential overexpression of *miR-130b* in CD133+ liver CSCs isolated from both HCC cell lines and freshly resected clinical samples[29]. The ecotpic expression of *miR-130b* was found to enhance chemoresistance, self-renewal ability *in vitro*, and tumorigenicity *in vivo* in CD133- cells, whereas the knockdown of *miR-130b* in CD133+ cells had the opposite effects. The direct downstream target of *miR-130b* was found to be the tumor suppressor gene *TP53INP1*, which is a pro-apoptotic stress-induced p53 target gene with both anti-proliferative and pro-apoptotic activities. Our study provided evidence that *miR-130b* can regulate CD133+ liver CSCs by silencing *TP53INP1*[29]. More recently, Liu *et al*.[30] have found that *miR-130b* (as well as *miR-15b*) was consistently and significantly up-regulated in HCC tissues, cell lines, and patient serum samples. Remarkably, the serum levels of these miRNAs were found to be significantly reduced after surgery, indicating that these circulating miRNAs originated from the tumor[30].

miR-150

miR-150 was also found to be preferentially expressed in CD133+ liver CSCs[31]. *miR-150* directly targeted c-Myb, and the overexpression of *miR-150* resulted in reductions in cell proliferation and spheroid formation and a significant decrease in CD133+ cells[31]. Moreover, the overexpression of *miR-150* led to the down-regulation of the cell cycle regulator *cyclin D1* and the cell survival regulator *Bcl-2*, resulting in cell cycle arrest and apoptosis in CD133+ cells[31]. The levels of circulating *miR-150* were also found to be at least 3-fold higher in HBV patients than in healthy individuals[28].

miR-145

miR-145 expression was found to be lower in HCC cancer stem cells derived from hepatocarcinoma cell line T3A-A3 than in the HCC cell line BEL-7402 or a normal liver sinusoidal endothelial cell line[32]. The restoration of *miR-145* in T3A-A3 cells resulted in senescence-like G_1 cell cycle arrest, the inhibition of colony and spheroid formation, and the inhibition of tumor formation in nude mice. *miR-145* overexpression led to a concomitant decrease in Oct4 expression, suggesting that *miR-145* exerts its tumor-suppressive effect in HCC via modulation of this stem cell marker[32]. To date, no study has reported the detection of *miR-145* in the serum of HCC patients.

miR-199a-3p

In addition to being deregulated in liver CSC subsets, miRNAs can directly target liver CSC markers, as reported by Henry *et al*.[33], who have found that *miR-199a-3p* targets CD44 and reduces the proliferation and invasive capabilities of CD44+ liver CSCs in HCC cell lines. The detection of *miR-199a-3p* in the serum of HCC patients has not been reported yet.

Clinical Implications

Chronic HBV and hepatitis C virus (HCV) infection are major risk factors for HCC development. Thus, differential miRNA patterns that can distinguish between infection and tumor stages will be of high value for the diagnosis of the disease. Differential miRNA expression patterns in CSC and non-CSC subsets may further provide useful prognostic information regarding response to adjuvant therapy and survival and recurrence rates. miRNAs are very stable both *in vitro* and *in vivo*, and miRNA expression can be easily detected even in formalin-fixed, paraffin-embedded sections[34]. Circulating miRNAs can also be easily detected in human serum and plasma[35]. Thus, in addition to the current diagnostic methods for HCC, the joint detection of endogenous and circulating miRNAs in tissue and serum/plasma may further enhance the sensitivity and specificity of disease diagnosis and prognosis.

miRNAs can function as either tumor suppressors (antagomirs) or tumor initiators (oncomirs). The possibility of targeting miRNAs for therapeutic use in treating cancer patients is now being tested through either increasing the expression of antagomirs or adding inhibitors to block oncomirs. For antagomirs, the use of the adeno-associated virus (AAV) system to overexpress *miR-26a* or *miR-122* in liver tumor-bearing tet-o-*MYC*;LAP-tTA mice was successful in inhibiting cancer cell growth, inducing tumor-specific apoptosis, and limiting disease progression without any toxic adverse effects[36,37]. The *in vivo* transfection of *miR-124* using liposomes has also been reported to be a feasible technique for restoring physiologic *miR-124* expression and suppressing HCC tumor growth, without any signs of toxicity, in a diethylnitrosamine (DEN)-treated tumor mice model[38]. For oncomirs, the administration of anti–*miR-221* oligonucleotides to *miR-221*–overexpressing transgenic mice and the administration of a cholesterol-modified isoform of anti–*miR-221* oligonucleotides in an orthotopic liver tumor xenograft model both resulted in a marked down-regulation of *miR-221* and liver cancer progression[39,40].

miR-130b is a prime example of an miRNA that is deregulated in tissue and serum samples of HCC patients as well as in liver CSC subsets. In this case, endogenous and circulating *miR-130b* in the tissue and serum samples of HCC patients can be used for diagnosis as well as to predict recurrence and evaluate treatment response. Alternatively, *miR-130b* can also be used as a direct therapeutic target.

Conclusion

The understanding of the role of miRNAs in liver CSCs remains limited to date. There is an increasing demand for further investigation of the use of miRNAs in liver CSC subsets. Together with a better understanding of the pathogenesis of HCC and the mechanisms underlying liver CSC subsets, we believe that miRNAs, which target multiple genes and pathways in the complex cancer environment, can be used as therapeutic targets and as diagnostic and/or prognostic tools in HCC. Further studies in this emerging field are warranted.

Acknowledgments

Liver cancer stem cell studies in the laboratory is generously supported by grants from the Research Grant Council—General Research Fund (HKU 760911M and HKU 773412M), Research Grant Council—Collaborative Research Fund (CUHK8/CRF/11R, HKU3/CRF/11R and HKU7/CRF/09), and the University of Hong Kong Strategic Research Theme in Cancer.

Received: 2013-02-27; revised: 2013-05-07;
accepted: 2013-05-07.

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