Editorial

circRNAs and miRNAs in the diagnosis of hepatocellular carcinoma: the long race to new targets

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The work by Yu and colleagues, recently published on the *Journal of Hepatology* (1) represents the latest step on a greatly debated and cherished argument: the mechanisms and clinical applicability of circular RNAs (circRNAs) and other non-coding RNAs (in primis microRNAs) in human tumoral disease, and in hepatocellular carcinoma (HCC) in particular. From their discovery in plants in 1976 no particular attention was attributed to the role of circRNAs in human, as they were considered as viroids (2). However, since 2012 we are experiencing a fast-growing interest towards circRNA functions in human: respectively 3 articles were published in 2013, 37 in 2015, 241 in 2017 and already 168 articles are available in May 2018 (source: https://www.ncbi.nlm.nih.gov/pubmed). circRNAs are very stable RNA structures, closed by a continuous loop, present in cell cytoplasm (3). They are highly represented in eukaryotic cells: up to now thousands of circRNAs have been identified in human. Among the circRNA functions, to "sponge" miRNAs is the most important: in this way circRNAs regulate miRNA concentration and—as a consequence—regulate the expression of several genes at transcriptional or post-transcriptional level (4).

In the very elegant study by Yu *et al.*, which included *in vitro* assays as well as animal and human models, the initial RNA-seq analysis performed on 5 HCC and 5 adjacent non-tumoral tissues identified 13,686 differentially expressed circRNAs. To identify the main key targets in HCC the authors filtered methodically the 13,686 detected circRNAs. Some of the main steps were the exclusion of the low abundance circRNAs (4,727 remaining) considering only novel circRNA. Then, through the comparison with known genes [2,322] encoding for circRNAs [4,142] and the Ingenuity Pathway Analysis, 236 circRNAs were identified. Based on their expression levels, the authors selected 16 circRNAs to be tested on 40 HCC and 40 control tissues; 10 circRNAs were validated (*Table 1*). In particular, the authors demonstrated *in vitro* that cSMARCA5 is an abundant, circular and stable transcript. Down-regulation of cSMARCA in HCC was elucidated throw *in vitro* and *in vivo* experiments: indeed, the DHX9 up-regulation in HCC prevents the formation of cSMARCA5. Finally, the downregulation of SMARCA5, studied in a new cohort of 163 patients, was associated with the tumor aggressiveness-growth and metastatic potential- and low survival rate. Low level of cSMARCA5 also induced the downregulation of the tumor-suppressor protein TIMP3; indeed overexpression of cSMARCA5 sponges the microRNAs miR-17-3p and miR-191b-5p (1). One additional point of interest is represented by the fact that miR-17-3p belongs to the miR-17-92 cluster, recently described as important for HCC resistance to systemic therapy with sorafenib (5).

This represents the last of a long series of recent findings about the association of circRNA-miRNA-mRNA and human neoplasms, as HCC (*Figure 1*). It was recently described how the hsa_circ_0003570 progressively decreased from normal liver to cirrhosis to HCC, with the lowest concentrations in high-grade HCCs with bigger dimensions (6). Most papers demonstrated that these circRNAs sponge at least one oncogenic or tumor-suppressor miRNA, also helping in the knowledge of miRNAs and other non-coding RNAs in the human tumor biology. For example, the up-regulation of hsa_circ_0000673 promotes the sponging

Table 1 Summary of the patients' cohort and workflow of the main analyses performed on tissues in the work by Yu *et al.* (1)

Cohort	Years	Phase	Number of patients	Tissue sampled	Main experimental assays	Number of circRNA	Number of circRNA selected
1	2016	Discovery	5	5 HCC and 5 adjacent noncancerous liver	RNA-seq	13,686 detected	16 (8 up and 8 down)
2	2016	Validation	40	40 HCC and 40 adjacent noncancerous liver	RT-qPCR, western blotting, IHC (DHX9, TIMP3 expression validation)	10 validated	1 (cSMARCA5)
3	2010–2011	Clinico-pathological associations	163	163 HCC and 163 adjacent noncancerous liver	RT-qPCR cSMARCA5 expression and outcome of patients	1	cSMARCA5
4	2016–2017	Liver fibrosis and inflammatory associations	37	37 healthy liver tissues	cSMARCA5 expression	1	cSMARCA5

HCC, hepatocellular carcinoma; IHC, immunohistochemistry.
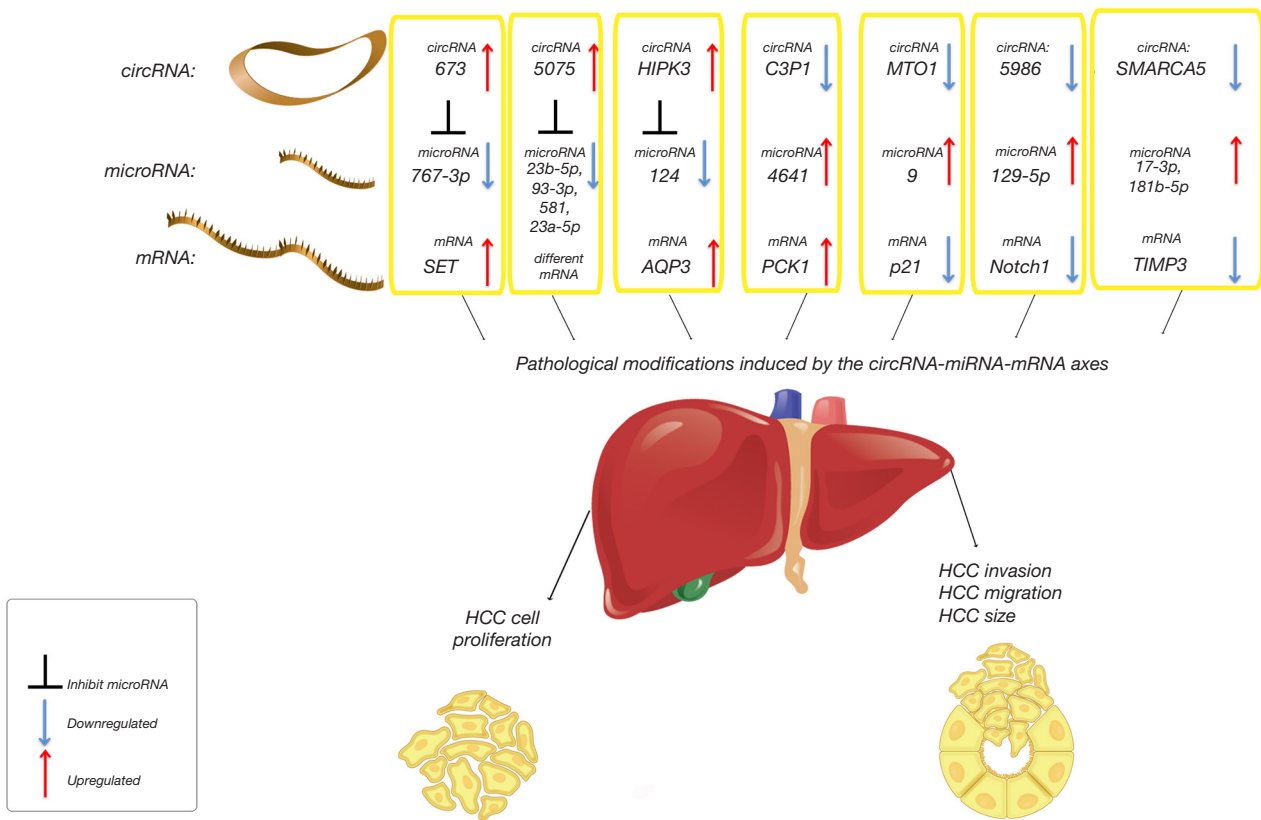
Figure 1 Recent findings in the deregulation of the circRNA-miRNA-mRNA axes in hepatocellular carcinoma. circRNA, circular RNA; HCC, hepatocellular carcinoma.

of miR-767-3p, bringing to a SET-mediated increase in HCC cell proliferation and invasion capability (7). Cdr1as showed oncogenic functions in HCC, by sponging miR-7 (8), hsa_circ_0005075 correlated with HCC tumor size, and sponged different miRNAs (3). Another study, *in vitro* and with animal model, found that circHIPK3 positively regulated the AQP3 gene via miR-124 sponging, promoting HCC cell proliferation and migration (9).

On the other hand, other circRNAs show tumor-suppressor activity: the down-regulation of circC3P1 promotes HCC progression and aggressiveness, decreasing the miR-4641 sponging and activating PCK1 (10). hsa_circRNA_0007874/hsa_circRNA_104135 (or mitochondrial translation optimization 1 homologue, circMTO1) suppresses HCC progression by sponging miR-9 and increasing p21 expression (11). Decreased hsa_circ_0005986 (with consequent increased miR-129-5p) in HCC was correlated with tumor dimensions, microvascular invasion and other pejorative prognostic factors (12).

The roles of circRNA are still largely unclear, and the current studies focus on their interaction with RNAs and protein: the functions of their secondary structures and their roles in DNA genome are not known. In literature, there is a huge growing number of new circRNAs involved in complex pathway interactions, most of them still unknown. However, the quick development of high-throughput RNA deep sequencing, along with the bioinformatics predictor tools, will enable a faster discovery of the pathways interactions of circRNAs (13). Considering their regulator properties, circRNAs might become very important for both diagnosis and therapeutic applications. For example, an *in vivo* increase in sponge expression is likely to provide an alternative to genetic knockouts of miRNAs. An advantage that cannot be neglected is that circRNA expression is highly tissue specific, quite the opposite to existing protein markers, that have low organ specificity: circRNAs—or the circRNA-miRNA axes—could be suitable diagnostic markers, with higher diagnostic sensitivity than traditional markers (14). circRNAs might become diagnostic biomarkers sooner than we expect, especially in HCC. Recently, a meta-analysis study including 1,752 patients with circRNA expression data from 17 publications showed that the expression of circRNA in HCC tissue was more prone to be detected than other tumor types with higher specificity and diagnostic value (15).

The study of circRNAs and miRNAs in human carcinogenesis is especially crucial in a multistep neoplasm such as HCC, in which most patients suffer from well-known pre-neoplastic conditions (i.e., cirrhosis), and the passage from regenerative and dysplastic nodules to openly

neoplastic lesion is well documented by the literature (16). In this setting, any possibility to set up a poorly invasive or not invasive early diagnosis of HCC is likely to change the natural history of cirrhotic patients, from prognosis after surgical resection or local therapy to priority in the transplant waiting list. Many efforts have been made in this direction in order to find serum RNA markers—especially serum miRNAs—for the early diagnosis of HCC. miR-122 alone or in combination with other miRNAs seemed the more promising (17), while other serum miRNAs were proposed in combination with alpha-fetoprotein (AFP) for the early diagnosis of HCC in cirrhotic patients (18). From this point of view, it would be interesting in the future to verify if cSMARCA5 levels in exosomes and blood plasma are also associated to HCC aggressiveness and survival rate as Yu and colleagues had demonstrated on tissue (1), and the same is applicable to any circRNA mentioned above. The possibility to regulate miRNAs such as the above-mentioned miR-17-3p is likely to open more perspective also in the response to sorafenib and other systemic therapies (5).

However, if we consider the clinical application of miRNAs, only a few of them have entered the preclinical and clinical stage. This is due in part to the lack of standardization during analysis (e.g., use of controls tissue, normalization methods) (19). Since today the majority of studies on circRNA mainly used the matched non-tumoral tissue as controls (15), but this changes from case to case. The field is still at his infancy and it would be beneficial to define from the beginning standard regulations for the analysis of circRNAs and miRNAs, especially in a tissue like liver, in which the "normality" itself is difficult to define, due to several changes incurring with age and chronic diseases.

In conclusion, the study of circRNAs and their interactions with miRNAs will be of great help in improving the knowledge about these non-coding RNAs, and the increasing interest of the scientific community on the argument seem to confirm that a panel of non-coding RNAs with clinical use will be available in commerce soon. As a matter of fact, looking at the gargantuan literature on the topic it seems strange that such panels have not been proposed yet for diagnosis and prognosis in human cancers. Apart from the crucial problem of finding standard controls, another limitation to the use of circRNAs as diagnostic tool is that the detection reagents tend to be expensive. However some promising new methods—such as bioluminescence or high-throughput sequencing—used in miRNA detection can be translated to circRNA detection (15). Thus a fast and cheap detection method for circRNA/miRNA would hopefully become a reality in the future, not only for research purposes but also for clinical use.

Footnote

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