Clinics and Research in Hepatology and Gastroenterology (2016) 40, 367—369

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

EDITORIAL

Hepatocellular carcinoma diagnosis: Circulating microRNAs emerge as robust biomarkers

Hepatocellular carcinoma (HCC) constitutes the second cause of death related to cancer worldwide, mainly due to a late diagnosis for advanced cancers, and a non-optimal detection for small tumors. This bad prognosis is also a consequence of the few therapeutic options available. It thus appears crucial to identify new biomarkers to improve HCC treatment and diagnosis. Nowadays, HCC detection is based on functional imaging, i.e. ultrasonography, magnetic resonance imaging or positron emission tomography. In addition, the measurement of α-fetoprotein (AFP) could also constitute an option for liver tumor diagnosis ([1] for a review). However, these techniques are not appropriate for the smallest tumors, and also show limited sensitivity and specificity. These recent years, microRNAs (miRNAs) have emerged as potent diagnostic and prognostic tools. These small non-coding RNAs were identified as key regulators of mRNA expression *via* translation inhibition and mRNA degradation ([2] for a review). They are key players of tumorigenesis, with either tumor suppressive or oncogenic activity, since 50% miRNAs are located in sites disrupted or amplified in cancer [3]. A number of reports devoted to miRNAs have highlighted these molecules as potent markers of tumor cells, with a microRNA signature identified for each type of cancerous lesion [4,5]. Concerning HCC, different studies agreed that these tumors are characterized by a loss of miR-122, Let-7 or miR-101, and inversely by an increase in miR-21, miR-221 and miR-224 level ([6] for a review). More interestingly, miRNAs could also be valuable non-invasive biomarkers in various biological fluids like serum, plasma, urine or saliva. Cell-free miRNAs could be associated to proteins like Argonaute 2, or lipid structures, either high- or low-density lipoproteins, but also encapsulated into membrane vesicles like microparticles or exosomes [7]. Hepatic exosomes are characterized by classical markers like CD81 and CD63, but could also incorporate other components required for liver-specific metabolic functions, i.e. in lipid, carbohydrate and xenobiotic metabolisms [8]. A growing number of reports have shown that hepatocellular carcinoma-derived exosomes could modify their growth, motility and response to treatment [9—11]. In addition, it has been shown that tumoral cells, from different tissular origin, secrete a larger amount of exosomes than non-tumoral ones, and that the materials incorporated, including miRNAs, could be modified [12]. Concerning circulating miRNAs in HCC, it has been described that miR-21, miR-221, miR-222 and miR-122 levels were deregulated in the serum of patients [13—15]. The reason why miR-122 is decreased in liver tumors and increased in patient sera is not elucidated yet. Regarding all these observations, efforts have been conducted these recent years to profile miRNAs detectable in patient serum, and which could be useful for HCC diagnosis. These studies have been enriched by two manuscripts, published this year in *Clinics and Research in Hepatology and Gastroenterology*, which demonstrate the potency of miRNA detection in serum to improve the diagnosis of early-stage and small tumors in particular.

These two studies have been performed on a significant cohort of tumors collected in China (50 HCC patients for Zhuang et al. and 112 for Lin et al.), and the robustness of miRNA-based diagnosis was assessed with serum AFP as a reference in both manuscripts. The study of Lin *et al.* focused on miR-224, a miRNA intensively studied in HCC in past. MiR-224 is described as an oncomiR in HCC, due to its pro-proliferative activity *via* AKT and SMAD4 deregulation [16,17], and its pro-migratory role [18]. MiR-224 has also been successfully detected in patient sera, and increases when HCC develops following hepatitis B virus (HBV) infection [19,20]. The present work of Lin et al. confirmed the induction of miR-224 in early-stage HBV-associated tumors as well as in serum. MiR-224 detection was thus a more

http://dx.doi.org/10.1016/j.clinre.2015.12.010

valuable diagnostic biomarker than AFP measurement. They also observed that the co-detection of AFP with miR-224 allows the discrimination of HCC from hepatitis B patients and healthy controls with the best accuracy as revealed by AUC (area under the ROC curve), sensitivity and specificity (>0,85, 87% and 76% in both cases, respectively). Interestingly, their data showed that this microRNA was produced from tumors, since its expression in serum was normalized after tumor resection.

The work of Zhuang et al. assessed the accuracy of circulating miR-21, miR-101 and miR-26a, three miRNAs deregulated in HCC, for HBV-associated tumor diagnosis. MiR-21 is described as upregulated in HCC tumors, while miR-101 and miR-26a are downregulated. These miRNAs control different aspects of HCC progression, i.e. cell proliferation, *via* EZH2 repression for miR-101 and miR-26a [17,21], or PTEN downregulation for miR-21 [22], apoptosis [23], and epithelial-to-mesenchymal transition [24]. The expression of these miRNAs is similarly deregulated in serum: miR-101 and miR-26a are impaired following HBV-associated HCC development [25,26], while miR-21 is upregulated in hepatitis B and C-induced HCC [27]. In their present study, Zhuang et al. corroborate the induction of miR-21 and the downregulation of miR-101 and miR-26a in the serum of HCC patients as compared to healthy donors and hepatitis patients. Surprisingly, they could show that miR-21 was efficiently impaired following tumor resection, while miR-101 and miR-26a expression was not modified after surgery. This suggests that some circulating miRNAs, such as miR-21, have a tumoral origin but others, like miR-101 and miR-26a, could emerge from non-tumoral cells. Nevertheless, they could promisingly show that the quantification of miR-101/miR-26a/miR-21 in serum is a better diagnosis tool than AFP alone. As described for miR-224 by the work of Lin *et al.*, they observed the best performance for the combination of these three miRNAs with AFP in terms of AUC, sensitivity and specificity as compared to healthy controls (AUC = 0.914, sensitivity 87%, specificity 78%). However, as mentioned this year by a paper of Sohn et al. [20], miR-21 was not significantly different between HCC patients and those affected by HBV. They thus successfully discriminate HCC from HBV patients according to miR-101/miR-26a only. Once again, the combination of these two miRNAs with AFP constitutes the best option (AUC = 0.854, sensitivity 72.5%, specificity 86.7%). Promisingly, when the authors focused their study on small tumors (<3 cm), they highlighted that the association of circulating miR-26a with AFP was reliable to differentiate HCC patients from those with hepatitis (AUC = 0.824, sensitivity 84%, specificity 76.7%). This is of great interest since, in case of small tumors, AFP measurement is not sufficient to discriminate small tumors from HBV patients. These results compare with those obtained by Lin et al. with the combination miR-224/AFP, which could successfully discriminate early-stage HCC from HBV patients (AUC = 0.87, sensitivity 87.5%, specificity 76.5%).

Altogether, these papers strongly support the great benefit of circulating miRNAs to detect HCC at very early stages, even if these encouraging data had to be confirmed on a larger cohort of samples. In particular, it appears crucial to explore if these data are specific of HBV-related HCC, or could be extended to hepatitis C virus (HCV), NASH or alcohol-associated HCC. To support this point, miR-101 level is decreased by HBx protein [28], and thus could be specific of this group of tumors, while miR-21 upregulation has been observed in HBV+ as well as on HCV+ HCC [27]. This clarification could lead to the identification of a panel of miRNAs, differentially deregulated according to HCC etiologies. This panel of miRNAs could be relevant for the diagnosis of each molecular subgroup of tumors, and could favor the administration of differential targeted therapies to patients. Although these two manuscripts agree that miRNA expression in serum is closely correlated to those observed in tumors, it remains to determine the precise cell origin of circulating miRNAs, since tumor resection did not restore the expression of all studied miRNAs. A better understanding could allow pathologists to support these miRNAs as potent biomarkers, i.e. in the case of relapse. Finally, despite the fact that AFP measurement is unsuccessful for early HCC diagnosis, it appears that a combination of AFP with miRNA detection presents the best accuracy in terms of sensitivity and specificity. Regarding to these two works, we may ask if a combination of miR-224 with miR-101/miR-26a and AFP would not constitute the optimal panel for early-stage HCC diagnosis.

To conclude, besides the therapeutic opportunities derived from miRNAs in a great number of cancers, these small molecules appear as very promising diagnosis and prognosis biomarkers, on the basis of their detection in serum but also in other body fluids like saliva or urine, and give a hope for improvement of HCC patient care in future.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Bellissimo F, Pinzone MR, Cacopardo B, Nunnari G. Diagnostic and therapeutic management of hepatocellular carcinoma. World J Gastroenterol 2015;21:12003—21.
[2] Pillai RS, Bhattacharyya SN, Filipowicz W. Repression of protein synthesis by miRNAs: how many mechanisms? Trends Cell Biol 2007;17:118—26.
[3] Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004;101:2999—3004.
[4] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834—8.
[5] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257—61.
[6] Gougelet A. MicroRNAs linking cancer and inflammation: focus on liver cancer. In: Babashah S, editor. microRNAs: key regulators of oncogenesis. Springer; 2014. p. 183—208.
[7] Turchinovich A, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. Nucleic Acids Res 2011;39:7223—33.
[8] Conde-Vancells J, Rodriguez-Suarez E, Embade N, Gil D, Matthiesen R, Valle M, et al. Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. J Proteome Res 2008;7:5157—66.

[9] Kogure T, Lin WL, Yan IK, Braconi C, Patel T. Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. Hepatology 2011;54:1237—48.
[10] Takahashi K, Yan IK, Kogure T, Haga H, Patel T. Extracellular vesicle-mediated transfer of long non-coding RNA ROR modulates chemosensitivity in human hepatocellular cancer. FEBS Open Bio 2014;4:458—67.
[11] He M, Qin H, Poon TC, Sze SC, Ding X, Co NN, et al. Hepatocellular carcinoma-derived exosomes promote motility of immortalized hepatocyte through transfer of oncogenic proteins and RNAs. Carcinogenesis 2015;36:1008—18.
[12] Braicu C, Tomuleasa C, Monroig P, Cucuianu A, Berindan-Neagoe I, Calin GA. Exosomes as divine messengers: are they the Hermes of modern molecular oncology? Cell Death Differ 2015;22:34—45.
[13] Xu J, Wu C, Che X, Wang L, Yu D, Zhang T, et al. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. Mol Carcinog 2011;50:136—42.
[14] Zhou J, Yu L, Gao X, Hu J, Wang J, Dai Z, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. J Clin Oncol 2011;29:4781—8.
[15] Li J, Wang Y, Yu W, Chen J, Luo J. Expression of serum miR-221 in human hepatocellular carcinoma and its prognostic significance. Biochem Biophys Res Commun 2011;406:70—3.
[16] Ma D, Tao X, Gao F, Fan C, Wu D. miR-224 functions as an onco-miRNA in hepatocellular carcinoma cells by activating AKT signaling. Oncol Lett 2012;4:483—8.
[17] Wang Y, Ren J, Gao Y, Ma JZ, Toh HC, Chow P, et al. MicroRNA-224 targets SMAD family member 4 to promote cell proliferation and negatively influence patient survival. PLoS One 2013;8:e68744.
[18] Li Q, Ding C, Chen C, Zhang Z, Xiao H, Xie F, et al. miR-224 promotion of cell migration and invasion by targeting Homeobox D 10 gene in human hepatocellular carcinoma. J Gastroenterol Hepatol 2014;29:835—42.
[19] Gao P, Wong CC, Tung EK, Lee JM, Wong CM, Ng IO. Deregulation of microRNA expression occurs early and accumulates in early stages of HBV-associated multistep hepatocarcinogenesis. J Hepatol 2011;54:1177—84.
[20] Sohn W, Kim J, Kang SH, Yang SR, Cho JY, Cho HC, et al. Serum exosomal microRNAs as novel biomarkers for hepatocellular carcinoma. Exp Mol Med 2015;47:e184.
[21] Wang L, Zhang X, Jia LT, Hu SJ, Zhao J, Yang JD, et al. c-Myc-mediated epigenetic silencing of MicroRNA-101 contributes to dysregulation of multiple pathways in hepatocellular carcinoma. Hepatology 2014;59:1850—63.
[22] Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007;133:647—58.
[23] Zhang H, Ozaki I, Mizuta T, Hamajima H, Yasutake T, Eguchi Y, et al. Involvement of programmed cell death 4 in transforming growth factor-beta1-induced apoptosis in human hepatocellular carcinoma. Oncogene 2006;25:6101—12.
[24] Zheng F, Liao YJ, Cai MY, Liu TH, Chen SP, Wu PH, et al. Systemic delivery of microRNA-101 potently inhibits hepatocellular carcinoma in vivo by repressing multiple targets. PLoS Genet 2015;11:e1004873.
[25] Fu Y, Wei X, Tang C, Li J, Liu R, Shen A, et al. Circulating microRNA-101 as a potential biomarker for hepatitis B virus-related hepatocellular carcinoma. Oncol Lett 2013;6:1811—5.
[26] Tan Y, Ge G, Pan T, Wen D, Chen L, Yu X, et al. A serum microRNA panel as potential biomarkers for hepatocellular carcinoma related with hepatitis B virus. PLoS One 2014;9:e107986.
[27] Amr KS, Ezzat WM, Elhosary YA, Hegazy AE, Fahim HH, Kamel RR. The potential role of miRNAs 21 and 199-a in early diagnosis of hepatocellular carcinoma. Gene 2016;575:66—70.
[28] Wei X, Xiang T, Ren G, Tan C, Liu R, Xu X, et al. miR-101 is down-regulated by the hepatitis B virus x protein and induces aberrant DNA methylation by targeting DNA methyltransferase 3A. Cell Signal 2013;25:439—46.

Angélique Gougelet [a,*,b,c]
Sabine Colnot [a,b,c]
[a] *Inserm, U1016, institut Cochin, 75014 Paris, France*
[b] *CNRS, UMR8104, 75014 Paris, France*
[c] *Université Paris Descartes, Sorbonne Paris Cité, 75006 Paris, France*

[*] Corresponding author.
E-mail address: angelique.gougelet@inserm.fr
(A. Gougelet)
Available online 26 January 2016