

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

MicroRNAs: New tools for diagnosis, prognosis and therapy in hepatocellular carcinoma?

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/126696> since

Published version:

DOI:10.1002/hep.26095

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This is the accepted version of the following article: [*MicroRNAs: New tools for diagnosis, prognosis and therapy in HCC?* Giordano S, Columbano A. *Hepatology*. **2013** Feb;57(2):840-7. doi: 10.1002/hep.26095. Epub 2012 Dec 26],

which has been published in final form at
[<http://onlinelibrary.wiley.com/doi/10.1002/hep.26095/full>]

MicroRNAs: new tools for diagnosis, prognosis and therapy in HCC?

Silvia Giordano¹ and Amedeo Columbano²

¹IRCC, Institute for Cancer Research at Candiolo, University of Torino Medical School, 10060 Candiolo (Torino), Italy; ²Department of Biomedical Sciences, Unit of Oncology and Molecular Pathology, University of Cagliari, Cagliari, Italy;

Key words:

HCC biomarkers; cancer surveillance; therapeutic targets, microRNA signatures.

Contact information:

Silvia Giordano MD, PhD
Department of Oncology
University of Torino, Medical school
Institute for Cancer Research and Treatment (IRCC)
Strada Provinciale 142
Candiolo (Torino), 10060, Italy
Phone + 39 0119933233
Fax +39 011 9933225
e-mail silvia.giordano@ircc.it

Amedeo Columbano, PhD
Department of Biomedical Sciences
Unit of Oncology and Molecular Pathology
University of Cagliari
Via Porcell 4, 09124 Cagliari, Italy
Phone: +39-070-6758345
Fax: +39-070-666062
e-mail: columbano@unica.it

Financial Support: this work was supported by Associazione Italiana Ricerca sul Cancro (AIRC, Grants IG-11821 to AC and IG-11819 to SG),

Acknowledgment: We apologize to all colleagues whose work could not be cited owing to space restriction. We thank our colleagues for useful discussion and F. Natale for editing the manuscript.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/hep.26095

ABSTRACT

MicroRNAs are evolutionary conserved small non-coding RNAs involved in the regulation of gene expression and protein translation. Many studies have shown that they play a crucial role in driving organ and tissue differentiation during embryogenesis and in the fine-tuning of fundamental biological processes, such as proliferation and apoptosis. Growing evidence indicates that their deregulation plays an important role in cancer onset and progression as well, where they act as oncogenes or oncosuppressors.

In this review we highlight the most recent findings on the role of microRNAs in hepatocellular carcinoma (HCC), by analyzing the possible mechanisms by which they contribute to this neoplasm. Moreover, we discuss the possible role of circulating miRNAs as biomarkers, a field that needs urgent improvement in the clinical surveillance of HCC, and the fascinating possibility of using them as therapeutic targets or drugs themselves.

Introduction

Hepatocellular carcinoma (HCC) is the third cause of cancer-related deaths worldwide. Multiple viruses, metabolic alterations leading to chronic inflammation, epigenetic and genetic changes cooperate in cancer development via a combination of common and distinct etiology specific pathways. Genome-wide gene expression microarray and quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) studies indicate a general aberrant activation of signalling pathways involved in cellular proliferation, survival, differentiation and angiogenesis, which are heterogeneously present in each HCC. However, what is missing is a signature or a single prominent characteristic pathway that defines this cancer.

Recently, it became clear that the classification and stratification of tumors can be performed also by evaluating the modulation of microRNAs (miRNAs), small non-coding RNAs which negatively control gene expression. Notably, microRNA expression profiles are able to classify tumors at different stages and to distinguish among subsets of patients with different molecular pathologies. Although changes in the expression of microRNAs between tumor specimens and the normal corresponding tissues have been investigated in HCC as well, the obtained results are often discordant and do not allow the identification of the microRNAs critical for development and progression of HCC (1). Furthermore, among the microRNAs whose expression has changed, several are probably altered not as a cause but as a consequence of the tumorigenic status.

In this review we summarize the main findings of the role of microRNAs in the context of liver cancer and discuss how this could help our understanding of the mechanisms underlying HCC development and progression and how they may improve diagnosis and treatment.

MicroRNAs and cancer

MicroRNAs are able to control gene expression at a post-transcriptional level, either by blocking mRNA translation or inducing their degradation. Thus, the mechanism of action of microRNAs has revolutionized the concept of gene expression regulation as we now know that mRNA levels in a cell do not strictly correlate with protein expression.

The involvement of microRNAs in cancer pathogenesis is well established as they can behave as oncogenes or tumor suppressor genes depending on the cellular function of their targets (2). Moreover, activation or suppression of specific microRNA families are mechanisms through which oncogenes, such as Myc, or tumor suppressor genes, such as p53, induce or inhibit tumorigenesis (3). Germline mutations have been detected in microRNA genes and in the binding sequences of target mRNAs. It is tempting to speculate that they might participate in familial predisposition to cancer, especially in those families where a culprit gene has not yet been identified (3,4). In addition, epigenetic modifications in microRNA loci, altering their transcription and affecting the metastatic ability of tumor cells have been described (5). The importance of microRNAs in cancer progression is also underlined by the observation that they can influence both the response to chemotherapy (6) and the development of drug resistance (7).

Remarkably, microRNAs might be very useful as cancer biomarkers since they are present in the blood and are very stable. Studies performed in preclinical models and in cancer patients demonstrated that cancer affects microRNA levels in the bloodstream and that specific microRNAs in the serum can be associated with specific tumors (4, 5). Even if this approach needs further validation, this discovery might open the path to an innovative way of detecting tumors by means of serum or plasma microRNA measurement.

Finally, tumor-associated microRNAs may represent a novel group of viable targets for therapeutic intervention. Further to this, studies attempting to translate work from the bench to the clinic are already well underway. The success obtained in lowering the level of plasma cholesterol in non-human primates by systemic administration of a microRNA inhibitor (6) gives hope for a possible application of a similar therapeutic approach in clinical oncology.

MicroRNAs and HCC

Many reports have shown microRNA deregulation in human HCCs. These works have either compared the cancer miRNome with that of non tumoral tissue or have studied specific microRNAs. The picture stemming from these investigations is not always super-imposable and this can be due to several technical issues. For example, studies have been performed using different techniques such as microarrays, RT-PCR-based assays and next generation sequencing. Even if reliable, they highlight significant differences that must be considered when analyzing results.

Using next generation sequencing, which provides data not only on quantitative alterations of the different microRNAs but also on their relative amount, Hou et al. (7) analysed the miRNomes of normal human liver and HCC. Interestingly, they found that around 86% of microRNAs were poorly expressed in normal liver, 13% were moderately expressed and less than 1% was abundantly expressed. The three most represented microRNAs were miR-122, miR-192 and miR 199 a/b-3p, accounting for 52%, 16.9% and 4.9% of the miRNome, respectively. Although a tumorigenic role of miR-122 has previously been described (8-12), Hou found that its expression decreased only in half of the HCCs and that it was poorly relevant for patients' survival; notably, he found a strong decrease of this microRNA only in viral-negative HCCs. Similarly, miR-192 did not seem to be significantly deregulated in HCC samples. On the other hand, deregulation of miR-199a/b 3p was observed in 40/40 patients, regardless of the underlying pathology, and its decrement significantly correlated with the poor survival of HCC patients. Among the moderately expressed microRNAs in HCCs and matched non-neoplastic tissues there were two families: let-7 and miR-100. The let -7 family was usually downregulated in HCC samples, even if an opposite behaviour was observed for let-7a and let-7f in viral-negative HCCs. Concerning the miR-100 family, both miR-100 and miR-99a were downregulated in HCCs of different etiology. The most upregulated microRNA was miR-21, which increased not only in the tumoral tissue, but also in the peritumoral non neoplastic tissue, compared to normal liver. This clearly shows that microRNA expression in the peritumoral non

neoplastic tissue (which is often used as a comparison) is frequently different from that of healthy “normal” liver. This may also be due to the presence in the cirrhotic peritumoral tissues of non-hepatocytic cells that express different sets of microRNAs. These findings may explain, at least in part, some of the discrepancies among different studies comparing HCC either to normal healthy liver or to non-neoplastic peritumoral tissue.

MicroRNA and molecular classifications of HCC

Profiling of human tumors based on microRNA expression has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment. In the attempt to use microRNAs to create a molecular classification of HCC, Murakami analyzed microRNA expression profiles in 25 pairs of HCC and adjacent non-tumorous tissue. He found that three microRNAs exhibited higher expression in the HCC samples, while five were downregulated (13). Classification of samples as HCC or normal, based on this data, provided an overall prediction accuracy of 97.8%. In addition, the expression levels of miR-92, miR-20 and miR-18 were inversely correlated with the degree of HCC differentiation. More recently, Toffanin et al. (14) performed a comprehensive genomic analysis by integrating microRNA data with gene expression analysis, copy number changes and assessment of cellular pathway activation by immunohistochemical and mutational analyses. They proposed a microRNA-based classification of 3 subclasses of HCC, displaying either activation of the Wnt pathway or enrichment of interferon-response-related genes or activation of IGF-1R and Akt-pathways.

Sato et al. (15) developed a mathematical model to assess the risk of HCC recurrence after liver resection, based on microRNA expression profiling. They found that the tumor microRNA profile could predict early recurrence, while the microRNA profile of the non tumoral tissue was predictive of late recurrence, suggesting that the tumor microRNA profile represents the malignant potential of primary tumors, associated with the presence of hepatic dissemination. The peritumoral

microRNA profile, instead, reflects the accumulation of genome abnormalities in the remaining non-cancerous liver cells, associated with multicentric *de novo* carcinogenesis.

Several studies examined the prognostic role of individual microRNAs in HCC, their mechanism of action and the biological effects resulting from their modulation in HCC cells; a list of the most relevant is provided in Supp. Table 1.

MicroRNA and metastasis

In several types of tumors, as well as in HCC, the analysis of microRNA expression has led to the identification of microRNAs promoting or repressing the metastatic process. Budhu et al. (16) identified a 20-microRNA tumor signature associated with HCC venous metastasis; this signature predicted survival and recurrence of HCC in patients with multinodular or solitary tumors, including those with early-stage disease. Moreover, it was an independent and significant predictor of patient prognosis, when compared to other available clinical parameters.

Contrasting results have recently been obtained by Wong et al. (17) who did not find differences in microRNA expression pattern between primary HCCs and venous metastases, but only a marked global reduction of microRNA expression levels in venous metastases, as compared with primary HCCs. Their data suggest that microRNA deregulation is a relatively early event in liver carcinogenesis and that the later global microRNA down-regulation aggravates the preexisting microRNA deregulation to further promote HCC metastasis.

Finally, some studies have analyzed the role of specific microRNAs in the metastatic process of HCC, identifying either pro-metastatic or antimetastatic microRNAs (Supp. Table 2).

MicroRNA and response to therapy

Works performed in many types of cancer have shown that microRNAs can influence the sensitivity of tumors to therapy. This notion holds true also for HCC (Supp. Table 3); restoration of miR-122 in HCC-cells makes them sensitive to adriamycin and vincristine through downregulation of MDR

related genes, the antiapoptotic gene Bcl-w and cyclin B1 (20). The same microRNA, as well as miR-199a-3p, were shown to affect sensitivity of HCC cells to doxorubicin (18, 19). DNA methylation of miR-193a-3p, instead, dictates 5-fluorouracil (5-FU) resistance of HCC cells via repression of the serine/arginine-rich splicing factor 2 which, in turn, up-regulates the proapoptotic splicing form of caspase 2 (23). Response to 5-FU was also studied by Tomimaru et al. (20) who found that HCC cells transfected with pre-miR-21 were resistant to IFN- α /5-FU, while cells expressing anti-miR-21 became sensitive to IFN- α /5-FU. Moreover, miR-21 expression in clinical HCC specimens was associated with the clinical response to the IFN- α /5-FU combination therapy and survival rate.

Many recent studies have demonstrated that resistance to chemotherapy is often due to altered expression of drug transporters. Indeed, up-regulation of Adenosine triphosphate-binding cassette (ABC) transporters in HCC occurs prior to chemotherapeutic treatment and is associated with microRNA down-regulation (25); up-regulation of five ABC genes in HCC patient samples appears to be mediated by 13 microRNAs.

Molecular therapies have recently entered the clinical scenario and have demonstrated good efficacy in other types of tumors, but resistance to treatment is usually observed in a short period of time. In HCC, where the most widely used biological therapies are interferon and sorafenib, the response to interferon was influenced by miR-146a, which induces resistance to treatment through its ability to downregulate SMAD4 (21), and by miR-26 whose low expression increases patients' response to interferon therapy (22). Zhou et al. (23) found that sorafenib, a small inhibitor of tyrosine and Raf kinases, recently approved for treatment of advanced HCC, altered the expression of 14 microRNAs; among these miRNAs, is miR-1274a that is up-regulated by sorafenib resulting in repression of ADAM9, a protease involved in sorafenib targeted-therapy of HCC. On the other hand, the liver-specific miR-122, frequently suppressed in primary HCCs, is able to sensitize HCC cells to sorafenib (29).

Circulating microRNAs and HCC

Efforts have been made to develop non-invasive serum biomarkers for the diagnosis of HCC,. Despite remarkable advances, the reliability of biomarkers such as alpha-fetoprotein (AFP) or des-g-carboxyprothrombin (DCP) is still debatable. Indeed, their specificity (in particular that of AFP) is low, especially in the context of chronic liver disease. Therefore, novel biomarkers for early HCC diagnosis are greatly needed. The finding that microRNAs can be detected in fluids like free microRNAs or contained within microvesicles, such as exosomes (membrane vesicles secreted by several cells) has opened new opportunities in the search for biomarkers in cancer. Indeed, the possibility of profiling microRNAs in circulation represents a non-invasive way to investigate disease-specific microRNAs and is an alternative and promising approach to current strategies for cancer surveillance (Supp. Table 4).

To determine whether serum or plasma levels of microRNAs have either a diagnostic or a prognostic value in human HCC, Li et al. (24) performed a study on 513 subjects. Serum microRNA expression profiling allowed the identification of 13 microRNAs that were differentially expressed in the sera of HBV+ patients and accurately discriminated not only HBV-associated HCCs from controls and HCV-associated HCCs, but also HBV-positive HCC cases from HBV cases. Moreover, 6 microRNAs were significantly upregulated in the sera of HBV-HCC patients. Interestingly, 2 of these microRNAs, namely miR-375 and miR-92a, were also present in the previous panel. The use of only 3 of these microRNAs (miR-25, miR-375, and let-7f) as biomarkers could separate HCC cases from controls. In addition, miR-375 alone had a ROC of 0.96 (specificity: 96%; sensitivity: 100%) in HCC prediction. Thus, this study demonstrates that serum microRNA profiles can serve as novel, non-invasive biomarkers for HBV-positive HCC diagnosis. More recently, Zhou et al. (25) also attempted to identify microRNAs for diagnosing HBV-related HCC. In comparison to the previous study, the analysis was performed on plasma rather than on serum. The study was conducted on 934 individuals and identified a microRNA panel providing a high diagnostic accuracy for HCC. The diagnostic performance of this panel did not depend on the

disease status and thus it seems to be of particular clinical value in diagnosing early stage HBV-related HCCs. Moreover, it could also differentiate HCC from healthy, chronic hepatitis B and cirrhosis. Remarkably, none of the microRNAs included in this panel coincided with those identified by Li et al. The reasons for the different results are not clear but may be related to different materials used (plasma vs. serum).

Other studies focused specifically on candidate microRNAs. Qi et al. (26) found that miR-122 in serum was higher in HCC patients than in healthy controls and that its levels were reduced in the post-operative serum samples. Why the expression of miR-122 was generally down-regulated in HCC, while its circulating levels increased in the same patients is unclear. One possibility is that the low level of this microRNA in tumor cells is due to increased release. However, this would not explain why high levels of miR-221 increased both in HCC as well as in the serum. Xu et al. (27) found that miR-122, miR-21 and miR-223 were high in the serum of patients with HCC but their levels, unlike those found by Zhou et al., could not discriminate between HCC and chronic hepatitis. In this regard, it is puzzling that while serum levels of miR-122 in Qi's work are up-regulated (as those found by Zhou et al. in the plasma), no such increase was observed in the serum by Li et al. Qu et al. (28)) investigated whether serum levels of miR-16, miR-195, and miR-199a, alone or in combination with conventional serum markers, could help differentiate HCC from chronic liver disease. They found that miR-16, as a single marker, had the highest sensitivity for HCC, followed by miR-199a, AFP, DCP, AFP-L3% and miR-195. As a second-line HCC marker, miR-16 yielded positive HCC predictions in 18 out of 26 HCC patients (most of which had a tumor size smaller than 3 cm) with negative results for all 3 conventional markers. Liu et al. (29) found that miR-15b, miR-21, miR-130b and miR-183 were highly expressed in 96 tumors and that their levels were markedly reduced after surgery, indicating the tumor-derived source of these circulating microRNAs. In a validation study, combined miR-15b and miR-130 yielded 98.2% sensitivity and 91.5% specificity. The detection sensitivity of the classifier in a subgroup of HCCs

with low AFP (<20 ng/ml) was 96.7% and the classifier also identified early-stage HCC cases that could not be detected by AFP.

Finally, expression of serum miR-221 was analyzed to investigate its prognostic value (30). High levels of miR-221 expression were correlated with tumor size, cirrhosis and tumor stage. In addition, Kaplan-Meier survival analysis showed that the overall survival rate of the high miR-221 expression group (27.6%) was significantly lower than that of the low miR-221 expression group (62.3%).

Altogether, these data show the feasibility of using circulating microRNAs as biomarkers for HCC diagnosis. At present, however, none of these studies have been translated into clinical practice. To fully uncover the clinical perspectives of this field of research, more work has to be done and some critical points, such as –for example – the best type of sample to be used (plasma, serum or urine) and appropriately-powered sample size, have to be carefully considered.

MicroRNAs as drugs or therapeutic targets

Many in vitro and preclinical studies have either reintroduced oncosuppressive microRNAs or inhibited oncogenic microRNAs in cancer cells, showing that these treatments often result in impairment of cell proliferation and invasion or in increased apoptosis. This implies that these microRNAs (in the case of oncosuppressors) or their inhibitors (in the case of oncogenic microRNAs) might be used as therapeutics. One of the advantages of modulating expression of microRNAs, as opposed to genes, resides in their ability to simultaneously target multiple genes and pathways. Moreover, targeting critical genes (and their related pathways) with more than one oncosuppressive microRNA could strongly enhance the biological efficacy and reduce the risk of resistance to therapy. On the other hand, the effect of microRNAs on gene expression could also result in clinically relevant side effects, due to off-target effects. The other major problem of microRNA-based anticancer therapies is their delivery. In the case of the reintroduction of oncosuppressive microRNAs into cancer cells, a system would be required in which microRNAs

could be delivered to all the tumor cells, otherwise untreated cells would sustain tumor recurrence. At present, such an efficient system of delivery is not available. Interestingly, as microRNAs can be exchanged between cells in paracrine manner, it is important to clarify if this natural biological mechanism could vicariate a relatively inefficient delivery. Figure 1 shows the most widely used strategies to target microRNAs in cancer.

As to HCC, many studies have shown that either exogenous expression of oncosuppressor microRNAs or inhibition of oncomiRs resulted in impaired growth or invasive ability of HCC cell lines *in vitro* or in xenografts. Furthermore, re-expression of a tumor suppressor microRNA could block cancer progression *in vivo* (37). Indeed, systemic administration of miR-26a in a mouse model of HCC using adeno-associated virus resulted in inhibition of cancer cell proliferation, induction of tumor-specific apoptosis and protection from disease progression without toxicity. This finding suggests that delivery of microRNAs may be an important therapeutic strategy.

Recently, two studies have shown the effectiveness of targeting miR-221 in HCC (31, 32). Both used systemic administration of either a cholesterol-modified isoform of miR-221 (33) or anti-miR-221 oligonucleotides (34) and observed an antitumoral effect, leading to prolonged mouse survival or a reduction in the number and size of tumor nodules. Furthermore, miR-124 administration inhibited and prevented DEN-induced HCC in mice, supporting the notion that systemic delivery of miR-124 may be a clinically viable anticancer therapeutic approach. This study also demonstrated that transient inhibition of HNF4a initiates hepatocellular transformation through a microRNA/inflammatory feedback loop circuit. As this circuit is perturbed in human HCCs, these data raise the possibility that the manipulation of this microRNA feedback-inflammatory loop has therapeutic potential for treating liver cancer.

Finally, Lanford et al. (35) showed that the liver-specific miR-122 is essential for HCV RNA accumulation in liver cells. They chronically treated HCV-infected chimpanzees with a miR-122 specific LNA oligonucleotide and observed suppression of viremia, without overt toxicity, thus

implying that miR-122 is essential for accumulation of HCV RNA in vivo. MiR-122 targeting may then represent a strategy to prevent the onset of chronic hepatitis, a major HCC risk factor.

Conclusions

Discovery of the critical role of microRNAs in modulating gene expression has not only changed our concept of gene expression regulation, but has also offered a new opportunity for designing anti-cancer strategies and therapies (Figure 2). However, it is essential to determine the safety of these treatments and to gain insight into the side-effects these therapies may have. Indeed, although our understanding of the role of microRNAs in cancer development is improving, it is still far from complete. Specifically, several relevant questions need to be solved (Table 1): i) are all microRNAs found deregulated in HCC critical for tumor development or is their deregulation simply the consequence of metabolic and structural rearrangements of fully transformed cancer cells? Studies should continue to focus on dissecting the carcinogenic process to identify microRNAs that are modified in the early phases of the process. The paucity of studies on HCC at the initial stages in humans is probably due to the clinical difficulty of diagnosing and collecting enough material to study early lesions. In this context, animal models of hepatocarcinogenesis, in which discrete lesions at different stages of progression can be identified and analyzed, will be extremely helpful. ii) Why do studies on microRNA profiling by different groups often fail to provide reproducible results and are frequently contradictory? Is this due to the intrinsic heterogeneity of human HCCs, to the different aetiological agents or to the type of technology used? New experimental strategies using system biology methodologies aimed at classifying and comparing the conditions underlying different studies by different groups are likely to provide an explanation to the apparently contradictory results and to help identify a precise microRNA signature in HCC. iii) The emergence of microRNAs as important regulators of metabolism has raised much interest not only from a scientific point of view but also from a clinical perspective. Indeed, a metabolic shift towards a resistant phenotype is almost invariably observed in preneoplastic and neoplastic cancer cells; while

therapeutic efforts to treat metabolic disorders have so far addressed ‘druggable’ targets, such as enzymes, the very recent finding that certain microRNAs may represent crucial regulators of metabolism raises the question of whether they may coordinately control metabolism as well. If so, targeting these mRNAs might impact on the metabolic machinery required for the resistant phenotype characteristic of the neoplastic cells. iv) The high stability of microRNAs in circulation makes them perfect biomarkers, especially for detection of early stage, pre-symptomatic diseases. However, the reason for the lack of correspondence between the levels of some microRNAs in HCC and in the patients’ fluids is still incompletely clear. Apart from these still unexplained findings, the fluid most reliable for the detection of microRNAs as possible cancer biomarkers has yet to be established before translation into clinical practice.

In view of the many unanswered questions, a greater understanding of the molecular mechanisms by which microRNAs regulate tumorigenesis is both a priority and a fascinating scientific challenge that may promote the development of innovative concepts in the diagnosis and treatment of cancer.

Reference List

1. Imbeaud S, Ladeiro Y, Zucman-Rossi J. Identification of novel oncogenes and tumor suppressors in hepatocellular carcinoma. *Semin Liver Dis* 2010;30:75-86.
2. Lujambio A, Lowe SW. The microcosmos of cancer. *Nature* 2012;482:347-355.
3. Lujambio A, Lowe SW. The microcosmos of cancer. *Nature* 2012;482:347-355.
4. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008;18:997-1006.
5. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A* 2008;105:10513-10518.
6. Elmen J, Lindow M, Schutz S, Lawrence M, Petri A, Obad S, et al. LNA-mediated microRNA silencing in non-human primates. *Nature* 2008;452:896-899.

7. Hou J, Lin L, Zhou W, Wang Z, Ding G, Dong Q, et al. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell* 2011;19:232-243.
8. Kutay H, Bai S, Datta J, Motiwala T, Pogribny I, Frankel W, et al. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. *J Cell Biochem* 2006;99:671-678.
9. Kutay H, Bai S, Datta J, Motiwala T, Pogribny I, Frankel W, et al. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. *J Cell Biochem* 2006;99:671-678.
10. Kutay H, Bai S, Datta J, Motiwala T, Pogribny I, Frankel W, et al. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. *J Cell Biochem* 2006;99:671-678.
11. Gramantieri L, Ferracin M, Fornari F, Veronese A, Sabbioni S, Liu CG, et al. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res* 2007;67:6092-6099.
12. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* 2009;28:3526-3536.
13. Murakami Y, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene* 2006;25:2537-2545.
14. Toffanin S, Hoshida Y, Lachenmayer A, Villanueva A, Cabellos L, Minguez B, et al. MicroRNA-based classification of hepatocellular carcinoma and oncogenic role of miR-517a. *Gastroenterology* 2011;140:1618-1628.
15. Sato F, Hatano E, Kitamura K, Myomoto A, Fujiwara T, Takizawa S, et al. MicroRNA profile predicts recurrence after resection in patients with hepatocellular carcinoma within the Milan Criteria. *PLoS ONE* 2011;6:e16435.
16. Budhu A, Jia HL, Forgues M, Liu CG, Goldstein D, Lam A, et al. Identification of metastasis-related microRNAs in hepatocellular carcinoma. *Hepatology* 2008;47:897-907.
17. Wong CM, Wong CC, Lee JM, Fan DN, Au SL, Ng IO. Sequential alterations of microRNA expression in hepatocellular carcinoma development and venous metastasis. *Hepatology* 2012;55:1453-1461.
18. Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, et al. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res* 2009;69:5761-5767.
19. Fornari F, Milazzo M, Chieco P, Negrini M, Calin GA, Grazi GL, et al. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res* 2010;70:5184-5193.
20. Tomimaru Y, Eguchi H, Nagano H, Wada H, Tomokuni A, Kobayashi S, et al. MicroRNA-21 induces resistance to the anti-tumour effect of interferon-alpha/5-fluorouracil in hepatocellular carcinoma cells. *Br J Cancer* 2010;103:1617-1626.

21. Tomokuni A, Eguchi H, Tomimaru Y, Wada H, Kawamoto K, Kobayashi S, et al. miR-146a suppresses the sensitivity to interferon-alpha in hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2011;414:675-680.
22. Ji J, Shi J, Budhu A, Yu Z, Forgues M, Roessler S, et al. MicroRNA expression, survival, and response to interferon in liver cancer. *N Engl J Med* 2009;361:1437-1447.
23. Zhou C, Liu J, Li Y, Liu L, Zhang X, Ma CY, et al. microRNA-1274a, a modulator of sorafenib induced a disintegrin and metalloproteinase 9 (ADAM9) down-regulation in hepatocellular carcinoma. *FEBS Lett* 2011;585:1828-1834.
24. Li LM, Hu ZB, Zhou ZX, Chen X, Liu FY, Zhang JF, et al. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res* 2010;70:9798-9807.
25. 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;29:4781-4788.
26. Qi P, Cheng SQ, Wang H, Li N, Chen YF, Gao CF. Serum microRNAs as biomarkers for hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. *PLoS ONE* 2011;6:e28486.
27. 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;50:136-142.
28. Qu KZ, Zhang K, Li H, Afdhal NH, Albitar M. Circulating microRNAs as biomarkers for hepatocellular carcinoma. *J Clin Gastroenterol* 2011;45:355-360.
29. Liu AM, Yao TJ, Wang W, Wong KF, Lee NP, Fan ST, et al. Circulating miR-15b and miR-130b in serum as potential markers for detecting hepatocellular carcinoma: a retrospective cohort study. *BMJ Open* 2012;2:e000825.
30. 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;406:70-73.
31. Park JK, Kogure T, Nuovo GJ, Jiang J, He L, Kim JH, et al. miR-221 silencing blocks hepatocellular carcinoma and promotes survival. *Cancer Res* 2011;71:7608-7616.
32. Callegari E, Elamin BK, Giannone F, Milazzo M, Altavilla G, Fornari F, et al. Liver tumorigenicity promoted by microRNA-221 in a mouse transgenic model. *Hepatology* 2012.
33. Park JK, Kogure T, Nuovo GJ, Jiang J, He L, Kim JH, et al. miR-221 silencing blocks hepatocellular carcinoma and promotes survival. *Cancer Res* 2011;71:7608-7616.
34. Callegari E, Elamin BK, Giannone F, Milazzo M, Altavilla G, Fornari F, et al. Liver tumorigenicity promoted by microRNA-221 in a mouse transgenic model. *Hepatology* 2012.
35. Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* 2010;327:198-201.

Legend to figures

Figure 1. Targeting microRNAs in cancer

Mature microRNAs are obtained from the primary transcript (pri-miRNA) through two sequential cleavages catalysed by two different RNA endonucleases, Drosha and Dicer respectively. The nascent pri-miRNA is first processed into a 70-nucleotide precursor called pre-miRNA; then the pre-miRNA is further cleaved to generate a 20-23 nucleotide mature microRNA. Depending on the degree of complementarity with the target sequence, microRNAs can hinder protein synthesis from a transcript either by interfering with the assembly of the ribosomes around the mRNA or by committing mRNAs to degradation through the activation of the RISC complex.

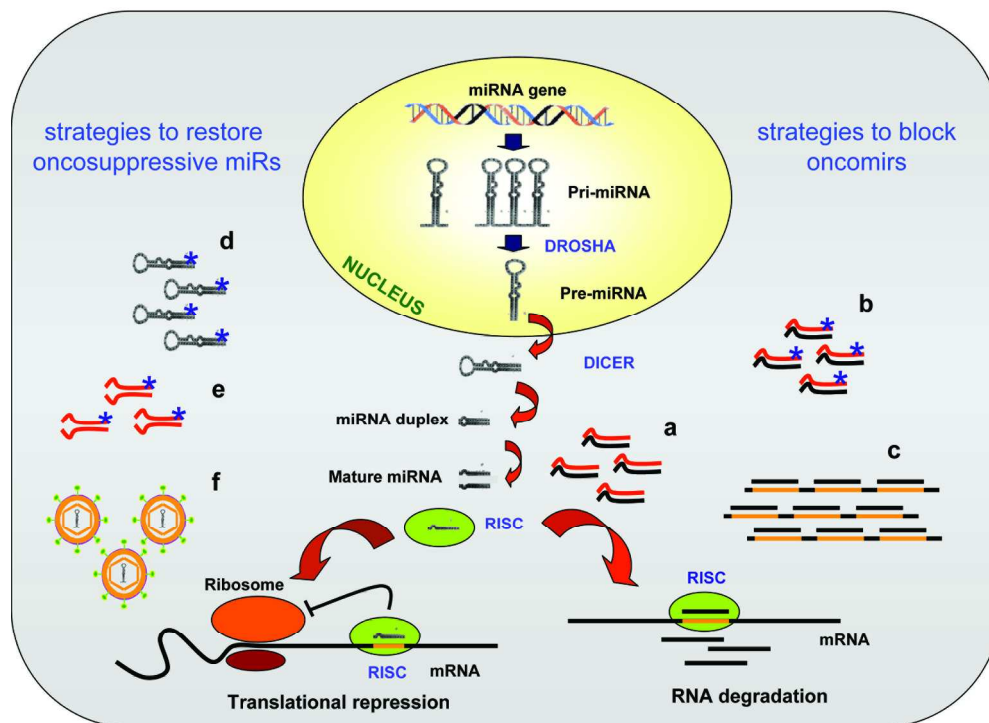
The most widely used strategies to block oncomirs in cancer (right side of the figure) are: (a) antisense oligonucleotides acting as competitive inhibitors of microRNAs; their major drawback is that they are quite unstable. (b) Locked nucleic acid (LNA) constructs showing high affinity for the target, high specificity and high aqueous solubility; (c) miRNA sponges which contain multiple binding sites for the microRNA of interest and act by competing with *bona fide* targets for microRNA binding. To restore oncosuppressive miRs (left side of the figure), either chemically modified miRNA mimics (d) or miRNA precursors (pre-miRNA) (e) have been developed. To improve their delivery and to have a long lasting expression, they can be incorporated into virus-like particles (mainly adenovirus-associated vectors) (f). *: Chemical modifications.

Figure 2. Potential use of microRNAs in HCC diagnosis, prognosis and treatment. Analysis of microRNAs in patients affected by HCC could be used for (i)

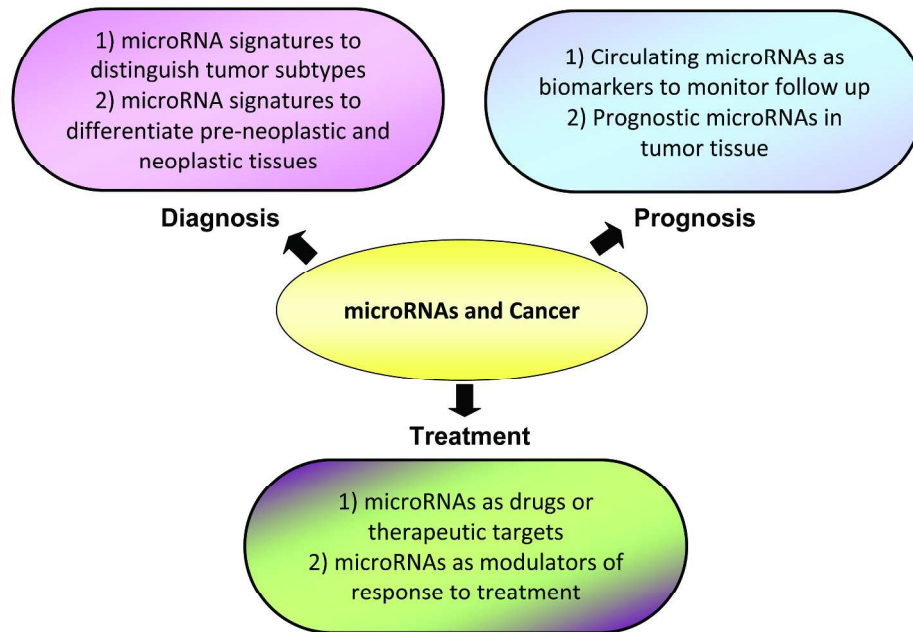
diagnostic purposes: (1) identification of tumor microRNA signatures which could help early diagnosis, by differentiating tumor subtypes; (2) identification of tumor microRNA signatures which could help early diagnosis, by differentiating small neoplastic lesions from the non tumoral tissue; *(ii) prognostic purposes:* (1) circulating microRNA profiling to identify relapse after treatment (2) identification of tumor microRNA signatures associated with different prognosis; *(iii) therapeutic purposes:* (1) inhibition of oncomiRs or restoration of oncosuppressive microRNAs (2) identification and modulation of microRNAs able to interfere with response to chemotherapy or to molecular therapy.

Table 1. Open questions

-
- ✓ Lack of studies on preneoplastic and early neoplastic lesions does not allow to discriminate which microRNAs are real drivers of the carcinogenic process.
 - Analysis of early tumor steps in humans
 - Use of animal models
 - ✓ Differences among the tumor signatures obtained by different groups
 - Heterogeneity of the pathology
 - Use of different technologies for the analysis
 - Use of different material as comparison (healthy liver vs. peritumoral tissue)
 - ✓ Differences among the signatures obtained by the analysis of circulating microRNAs
 - Starting material (plasma vs. serum)
 - Heterogeneity of the pathology
 - Use of different technologies for the analysis
 - ✓ Use of microRNAs as drugs or drug targets
 - Efficiency of delivery
 - Persistency of response
 - Side effects
-



257x190mm (300 x 300 DPI)



254x190mm (300 x 300 DPI)

Supporting Table 1. Prognostic microRNAs in human HCC

microRNA	Main finding	Reference
miR-182	miR overexpression in HCC correlates with intrahepatic metastasis and poor prognosis	Wang, <i>et al.</i> 2012 (1)
miR-155, miR-15a, miR-432, miR-486-3p, miR-15b, miR-30b	High expression levels of these miRs are significantly associated with RFS	Huang YH, <i>et al.</i> 2012 (2)
6 miR signature	Significant independent predictor of overall survival and recurrence-free survival	Han, <i>et al.</i> 2012 (3)
miR-10b	High miR-10b is an independent predictor of poor prognosis	Li, <i>et al.</i> 2012 (4)
C19CM	High C19CM miRNA levels correlate with poor clinico-pathological features, increased Risk of recurrence and shorter overall survival	Augello, <i>et al.</i> 2012 (5)
miR21, miR221	Up-regulation is associated with tumor stage and poor prognosis	Karakatsanis, <i>et al.</i> 2011 (6)
miR-155	High levels in patients with post-OLT recurrence and correlation with micro-vascular invasion. Independent predictor of poor prognosis.	Han, <i>et al.</i> 2012 (7)
67 miRs signature	Discriminates patients with HCC recurrence after transplant from those without recurrence	Barry, <i>et al.</i> 2012 (8)
miR-135a	High miR-135a expression correlates with low OS and DFS	Liu, <i>et al.</i> 2012 (9)
miR-99a	Low miR-99a correlates with shorter survival and is an independent prognostic predictor	Li, <i>et al.</i> 2011 (10)
miR-203	High miR-203 correlates with better RFS and OS. High miR-203 expression is an independent predictor of good prognosis	Chen, <i>et al.</i> 2012 (11)
DLK1-DIO3 miRNA cluster	Upregulation of this cluster is associated with a stem-cell-like phenotype and poor prognosis	Luk, <i>et al.</i> 2011 (12)
miR-124	Low expression is significantly associated with a more aggressive phenotype and poor prognosis	Zheng, <i>et al.</i> 2012 (13)
miR-199b-5p	Low expression is associated with poor OS and PFS	Wang, <i>et al.</i> 2011 (14)
miR signatures in tumor and non-tumor tissues	Tumor signature tends to predict early recurrence; non-tumor tissue signature tends to predict late recurrence.	Sato, <i>et al.</i> 2011 (15)
miR-139	Downregulation of miR-139 is associated with poor prognosis and features of metastatic tumors	Wong, <i>et al.</i> 2011 (16)
miR-22	Low miR-22 expression is predictive of poor survival	Zhang, <i>et al.</i> 2010 (17)
let-7g	Low let-7g expression is predictive of poor survival	Ji, <i>et al.</i> 2010 (18)
miR-29	miR-29 downregulation is associated with worse DFS	Xiong, <i>et al.</i> 2010 (19)
miR-122	miR-122 is downregulated in tumors characterized by poor prognosis	Coulouarn <i>et al.</i> 2009 (20)

RFS= Recurrence Free Survival; OS= Overall survival; DFS= Disease Free Survival; PFS= Progression Free Survival; OLT= Orthotopic Liver Transplantation.

Reference List

1. Wang J, Li J, Shen J, Wang C, Yang L, Zhang X. MicroRNA-182 downregulates metastasis suppressor 1 and contributes to metastasis of hepatocellular carcinoma. *BMC Cancer* 2012;12:227.
2. Huang YH, Lin KH, Chen HC, Chang ML, Hsu CW, Lai MW, et al. Identification of postoperative prognostic microRNA predictors in hepatocellular carcinoma. *PLoS One* 2012;7:e37188.
3. Han ZB, Zhong L, Teng MJ, Fan JW, Tang HM, Wu JY, et al. Identification of recurrence-related microRNAs in hepatocellular carcinoma following liver transplantation. *Mol Oncol* 2012;6:445-457.
4. Li QJ, Zhou L, Yang F, Wang GX, Zheng H, Wang DS, et al. MicroRNA-10b promotes migration and invasion through CADM1 in human hepatocellular carcinoma cells. *Tumour Biol* 2012.
5. Augello C, Vaira V, Caruso L, Destro A, Maggioni M, Park YN, et al. MicroRNA profiling of hepatocarcinogenesis identifies C19MC cluster as a novel prognostic biomarker in hepatocellular carcinoma. *Liver Int* 2012;32:772-782.
6. Karakatsanis A, Papaconstantinou I, Gazouli M, Lyberopoulou A, Polymeneas G, Voros D. Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. *Mol Carcinog* 2011.
7. Han ZB, Chen HY, Fan JW, Wu JY, Tang HM, Peng ZH. Up-regulation of microRNA-155 promotes cancer cell invasion and predicts poor survival of hepatocellular carcinoma following liver transplantation. *J Cancer Res Clin Oncol* 2012;138:153-161.
8. Barry CT, D'Souza M, McCall M, Safadjou S, Ryan C, Kashyap R, et al. Micro RNA expression profiles as adjunctive data to assess the risk of hepatocellular carcinoma recurrence after liver transplantation. *Am J Transplant* 2012;12:428-437.
9. Liu S, Guo W, Shi J, Li N, Yu X, Xue J, et al. MicroRNA-135a contributes to the development of portal vein tumor thrombus by promoting metastasis in hepatocellular carcinoma. *J Hepatol* 2012;56:389-396.
10. Li D, Liu X, Lin L, Hou J, Li N, Wang C, et al. MicroRNA-99a inhibits hepatocellular carcinoma growth and correlates with prognosis of patients with hepatocellular carcinoma. *J Biol Chem* 2011;286:36677-36685.
11. Chen HY, Han ZB, Fan JW, Xia J, Wu JY, Qiu GQ, et al. miR-203 expression predicts outcome after liver transplantation for hepatocellular carcinoma in cirrhotic liver. *Med Oncol* 2012;29:1859-1865.

12. Luk JM, Burchard J, Zhang C, Liu AM, Wong KF, Shek FH, et al. DLK1-DIO3 genomic imprinted microRNA cluster at 14q32.2 defines a stemlike subtype of hepatocellular carcinoma associated with poor survival. *J Biol Chem* 2011;286:30706-30713.
13. Zheng F, Liao YJ, Cai MY, Liu YH, Liu TH, Chen SP, et al. The putative tumour suppressor microRNA-124 modulates hepatocellular carcinoma cell aggressiveness by repressing ROCK2 and EZH2. *Gut* 2012;61:278-289.
14. Wang C, Song B, Song W, Liu J, Sun A, Wu D, et al. Underexpressed microRNA-199b-5p targets hypoxia-inducible factor-1alpha in hepatocellular carcinoma and predicts prognosis of hepatocellular carcinoma patients. *J Gastroenterol Hepatol* 2011;26:1630-1637.
15. Sato F, Hatano E, Kitamura K, Myomoto A, Fujiwara T, Takizawa S, et al. MicroRNA profile predicts recurrence after resection in patients with hepatocellular carcinoma within the Milan Criteria. *PLoS ONE* 2011;6:e16435.
16. Wong CC, Wong CM, Tung EK, Au SL, Lee JM, Poon RT, et al. The microRNA miR-139 suppresses metastasis and progression of hepatocellular carcinoma by down-regulating Rho-kinase 2. *Gastroenterology* 2011;140:322-331.
17. Zhang J, Yang Y, Yang T, Liu Y, Li A, Fu S, et al. microRNA-22, downregulated in hepatocellular carcinoma and correlated with prognosis, suppresses cell proliferation and tumorigenicity. *Br J Cancer* 2010;103:1215-1220.
18. Ji J, Zhao L, Budhu A, Forgues M, Jia HL, Qin LX, et al. Let-7g targets collagen type I alpha2 and inhibits cell migration in hepatocellular carcinoma. *J Hepatol* 2010;52:690-697.
19. Xiong Y, Fang JH, Yun JP, Yang J, Zhang Y, Jia WH, et al. Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. *Hepatology* 2010;51:836-845.
20. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* 2009;28:3526-3536.

Supporting Table 2. MicroRNAs and metastasis

MicroRNA	Main finding	Effector	Reference
miR-7	Inhibits metastasis in vitro and in vivo	PI3K/AKT/mTOR pathway	Fang <i>et al</i> ; 2012 (1)
miR-210	Up-regulated in HCC. Induced by hypoxia. Increases migration and invasion	Vacuole membrane Protein 1 (VMPI)	Ying <i>et al</i> ; 2011 (2)
miR-1395p, miR-101 miR-125b, let-7c, miR-200b	Downregulated in HCC with metastatic features Negatively regulate HCC metastasis	miRs epigenetically silenced by EZH2	Au <i>et al</i> ; 2012 (3)
miR-21	Promotes migration and invasion of HCC cells	Up-regulated in HCC. Controls Programmed cell death 4 (PDCD4)	Zhu <i>et al</i> ; 2012 (4)
miR-96	Promotes migration and invasion of HCC cells	Controls osteopontin levels	Chen <i>et al</i> ; 2012 (5)
Global miRNA analysis	Global reduction of miRNA expression levels in venous metastases vs. primary HCCs		Wong <i>et al</i> ; 2012 (6)
miR-155	High levels in tumor tissues with post-OLT HCC recurrence and correlation with micro-vascular invasion. Promotes invasion of HCC cells.		Han <i>et al</i> ; 2012 (7)
miR-135a	Favors invasion and metastasis in vitro. In vivo blockade reduces the development of portal vein tumor thrombi	miR-135a is transcribed by FOXM1 and controls metastasis suppressor 1	Liu <i>et al</i> ; 2012 (8)
miR-29b	Downregulation associated with poor recurrence-free survival. Suppresses proangiogenic and invasive ability of HCC cells	Directly controls MMP2	Fang <i>et al</i> ; 2011 (9)
miR-124	Reduced expression associated with poor prognosis. Re-expression in HCC cells inhibits invasion in vitro and metastasis in vivo.	Targets ROCK2 and EZH2	Zheng <i>et al</i> ; 2012.(10)
miR-338-3p	Decreased in aggressive HCC. Re-expression suppresses invasion of HCC cells	Targets Smoothened	Huang <i>et al</i> ; 2011 (11)
miR-198	Downregulated in HCC. Re-expression suppresses HGF-induced invasion of HCC cells	Targets c-MET	Tan <i>et al</i> ; 2011 (12)

miR-200, miR-192	Transactivated by p53. Regulate Epithelial mesenchymal transition	miR-200 targets ZEB 1 / 2, miR-192 targets ZEB 2	Kim <i>et al</i> ; 2011 (13)
miR-139	Reduced expression associated with poor prognosis. Re-expression in HCC cells inhibits invasion in vitro and metastasis in vivo.	Targets ROCK2	Wong <i>et al</i> ; 2011 (14)
miR-125b	Suppresses HCC cell growth in vitro and in vivo and inhibits invasion	Targets LIN28B	Liang <i>et al</i> ; 2010 (15)
miR-151-5p	Frequently amplified in HCC. Correlated with intrahepatic metastases. Increases HCC invasion in vitro and in vivo	Targets Rho GDIA. It synergizes with its host gene FAK	Ding <i>et al</i> ; 2010 (16)
Let-7g	Low in metastatic HCC. Ectopic expression inhibits HCC migration and growth	Targets COL1A2	Ji <i>et al</i> ; 2010 (17)
miR-17-5p	Overexpressed in HCC. Up-regulates migration and proliferation of HCC cells	Activates p38 MAPK pathway and Promotes HSP27 phosphorylation	Yang <i>et al</i> ; 2010 (18)
miR-30d	Reduced expression associates with poor prognosis. Re-expression in HCC cells inhibits invasion in vitro and metastasis in vivo.	Targets Galphai2	Yao <i>et al</i> ; 2010 (19)
miR-122	Reduced expression associated with poor prognosis. Re-expression in HCC cells inhibits invasion in vitro.	miR-122 is under the transcriptional control of HNF1A, HNF3A, HNF3B	Coulouarn <i>et al</i> ; 2009 (20)
miR-23b	miR expression in HCC cells decreases migration and proliferation	Targets uPA and c-MET	Salvi <i>et al</i> ; 2009 (21)
miR-143	Increased in metastatic HCC. Promotes invasive and metastatic behavior in vitro and in vivo.	Targets fibronectin type III domain containing 3B	Zhang <i>et al</i> ; 2009 (22)
miR-34a	Downregulated in HCC. Ectopic expression inhibits Met-dependent cell migration and invasion.	c-Met	Li <i>et al</i> ; 2009 (23)
20 miRs metastasis signature	Significantly predicts primary HCCs with venous metastases from metastasis-free solitary tumors		Budhu <i>et al</i> ; 2008 (24)

Reference List

1. Fang Y, Xue JL, Shen Q, Chen J, Tian L. MicroRNA-7 inhibits tumor growth and metastasis by targeting the phosphoinositide 3-kinase/Akt pathway in hepatocellular carcinoma. *Hepatology* 2012;55:1852-1862.
2. Ying Q, Liang L, Guo W, Zha R, Tian Q, Huang S, et al. Hypoxia-inducible microRNA-210 augments the metastatic potential of tumor cells by targeting vacuole membrane protein 1 in hepatocellular carcinoma. *Hepatology* 2011;54:2064-2075.
3. Au SL, Wong CC, Lee JM, Fan DN, Tsang FH, Ng IO, et al. Enhancer of zeste homolog 2 epigenetically silences multiple tumor suppressor microRNAs to promote liver cancer metastasis. *Hepatology* 2012;56:622-631.
4. Zhu Q, Wang Z, Hu Y, Li J, Li X, Zhou L, et al. miR-21 promotes migration and invasion by the miR-21-PDCD4-AP-1 feedback loop in human hepatocellular carcinoma. *Oncol Rep* 2012;27:1660-1668.
5. Chen RX, Xia YH, Xue TC, Ye SL. Suppression of microRNA-96 expression inhibits the invasion of hepatocellular carcinoma cells. *Mol Med Report* 2012;5:800-804.
6. Wong CM, Wong CC, Lee JM, Fan DN, Au SL, Ng IO. Sequential alterations of microRNA expression in hepatocellular carcinoma development and venous metastasis. *Hepatology* 2012;55:1453-1461.
7. Han ZB, Chen HY, Fan JW, Wu JY, Tang HM, Peng ZH. Up-regulation of microRNA-155 promotes cancer cell invasion and predicts poor survival of hepatocellular carcinoma following liver transplantation. *J Cancer Res Clin Oncol* 2012;138:153-161.
8. Liu S, Guo W, Shi J, Li N, Yu X, Xue J, et al. MicroRNA-135a contributes to the development of portal vein tumor thrombus by promoting metastasis in hepatocellular carcinoma. *J Hepatol* 2012;56:389-396.
9. Fang JH, Zhou HC, Zeng C, Yang J, Liu Y, Huang X, et al. MicroRNA-29b suppresses tumor angiogenesis, invasion, and metastasis by regulating matrix metalloproteinase 2 expression. *Hepatology* 2011;54:1729-1740.
10. Zheng F, Liao YJ, Cai MY, Liu YH, Liu TH, Chen SP, et al. The putative tumour suppressor microRNA-124 modulates hepatocellular carcinoma cell aggressiveness by repressing ROCK2 and EZH2. *Gut* 2012;61:278-289.

11. Huang XH, Chen JS, Wang Q, Chen XL, Wen L, Chen LZ, et al. miR-338-3p suppresses invasion of liver cancer cell by targeting smoothened. *J Pathol* 2011;225:463-472.
12. Tan S, Li R, Ding K, Lobie PE, Zhu T. miR-198 inhibits migration and invasion of hepatocellular carcinoma cells by targeting the HGF/c-MET pathway. *FEBS Lett* 2011;585:2229-2234.
13. Kim T, Veronese A, Pichiorri F, Lee TJ, Jeon YJ, Volinia S, et al. p53 regulates epithelial-mesenchymal transition through microRNAs targeting ZEB1 and ZEB2. *J Exp Med* 2011;208:875-883.
14. Wong CC, Wong CM, Tung EK, Au SL, Lee JM, Poon RT, et al. The microRNA miR-139 suppresses metastasis and progression of hepatocellular carcinoma by down-regulating Rho-kinase 2. *Gastroenterology* 2011;140:322-331.
15. Liang L, Wong CM, Ying Q, Fan DN, Huang S, Ding J, et al. MicroRNA-125b suppressed human liver cancer cell proliferation and metastasis by directly targeting oncogene LIN28B2. *Hepatology* 2010;52:1731-1740.
16. Ding J, Huang S, Wu S, Zhao Y, Liang L, Yan M, et al. Gain of miR-151 on chromosome 8q24.3 facilitates tumour cell migration and spreading through downregulating RhoGDI. *Nat Cell Biol* 2010;12:390-399.
17. Ji J, Zhao L, Budhu A, Forgues M, Jia HL, Qin LX, et al. Let-7g targets collagen type I alpha2 and inhibits cell migration in hepatocellular carcinoma. *J Hepatol* 2010;52:690-697.
18. Yang F, Yin Y, Wang F, Wang Y, Zhang L, Tang Y, et al. miR-17-5p Promotes migration of human hepatocellular carcinoma cells through the p38 mitogen-activated protein kinase-heat shock protein 27 pathway. *Hepatology* 2010;51:1614-1623.
19. Yao J, Liang L, Huang S, Ding J, Tan N, Zhao Y, et al. MicroRNA-30d promotes tumor invasion and metastasis by targeting Galphai2 in hepatocellular carcinoma. *Hepatology* 2010;51:846-856.
20. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* 2009;28:3526-3536.

21. Salvi A, Sabelli C, Moncini S, Venturin M, Arici B, Riva P, et al. MicroRNA-23b mediates urokinase and c-met downmodulation and a decreased migration of human hepatocellular carcinoma cells. *FEBS J* 2009;276:2966-2982.
22. Zhang X, Liu S, Hu T, Liu S, He Y, Sun S. Up-regulated microRNA-143 transcribed by nuclear factor kappa B enhances hepatocarcinoma metastasis by repressing fibronectin expression. *Hepatology* 2009;50:490-499.
23. Li N, Fu H, Tie Y, Hu Z, Kong W, Wu Y, et al. miR-34a inhibits migration and invasion by down-regulation of c-Met expression in human hepatocellular carcinoma cells. *Cancer Lett* 2009;275:44-53.
24. Budhu A, Jia HL, Forgues M, Liu CG, Goldstein D, Lam A, et al. Identification of metastasis-related microRNAs in hepatocellular carcinoma. *Hepatology* 2008;47:897-907.

Supporting Table 3. MicroRNAs modulating therapeutic response

MicroRNA	Finding	Mediator	Reference
miR-122	Increases sensitivity to doxorubicin	NS	Fornari <i>et al</i> ; 2009 (1)
miR-199a-3p	Restoring miR decreased levels increases sensitivity to doxorubicin-induced apoptosis	modulation of mTOR and c-MET	Fornari <i>et al</i> ; 2010 (2)
miR-122	Sensitizes HCC cells to Adriamycin and vincristine	downregulates MDR-related genes Bcl-w and cyclin B1	Xu <i>et al</i> ; 2011 (3)
miR-193a-3p	Promotes resistance to 5-FU	decreases SRSF2	Ma <i>et al</i> ; 2012 (4)
miR-122	Sensitizes HCC cells to sorafenib	NS	Bai <i>et al</i> ; 2009 (5)
miR-193b	Decreases the IC(50) to sorafenib	decreases Mcl-1 expression	Braconi <i>et al</i> ; 2010 (6)
miR-1274a	Up-regulated by sorafenib mediates resistance to this drug	downregulates ADAM9	Zhou <i>et al</i> ; 2011 (7)
miR-21	Induces resistance to interferon- α /5FU in HCC cells	PTEN and PDCD4	Tomimaru <i>et al</i> ; 2010 (8)
miR-146a	Suppresses sensitivity to interferon- α in HCC cells	downregulates SMAD4	Tomokuni <i>et al</i> ; 2011 (9)
13 miRs	Mediate multidrug resistance	downregulate ABC (Adenosine Triphosphate Binding Cassette) transporters	Borel <i>et al</i> ; 2012 (10)

NS = not specified; 5-FU= 5- Fluorouracil

Reference List

1. Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, et al. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res* 2009;69:5761-5767.
2. Fornari F, Milazzo M, Chieco P, Negrini M, Calin GA, Grazi GL, et al. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res* 2010;70:5184-5193.
3. Xu Y, Xia F, Ma L, Shan J, Shen J, Yang Z, et al. MicroRNA-122 sensitizes HCC cancer cells to adriamycin and vincristine through modulating expression of MDR and inducing cell cycle arrest. *Cancer Lett* 2011;310:160-169.
4. Ma K, He Y, Zhang H, Fei Q, Niu D, Wang D, et al. DNA methylation-regulated miR-193a-3p dictates resistance of hepatocellular carcinoma to 5-fluorouracil via repression of SRSF2 expression. *J Biol Chem* 2012;287:5639-5649.
5. Bai S, Nasser MW, Wang B, Hsu SH, Datta J, Kutay H, et al. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *J Biol Chem* 2009;284:32015-32027.
6. Braconi C, Valeri N, Gasparini P, Huang N, Taccioli C, Nuovo G, et al. Hepatitis C virus proteins modulate microRNA expression and chemosensitivity in malignant hepatocytes. *Clin Cancer Res* 2010;16:957-966.
7. Zhou C, Liu J, Li Y, Liu L, Zhang X, Ma CY, et al. microRNA-1274a, a modulator of sorafenib induced a disintegrin and metalloproteinase 9 (ADAM9) down-regulation in hepatocellular carcinoma. *FEBS Lett* 2011;585:1828-1834.
8. Tomimaru Y, Eguchi H, Nagano H, Wada H, Tomokuni A, Kobayashi S, et al. MicroRNA-21 induces resistance to the anti-tumour effect of interferon-alpha/5-fluorouracil in hepatocellular carcinoma cells. *Br J Cancer* 2010;103:1617-1626.
9. Tomokuni A, Eguchi H, Tomimaru Y, Wada H, Kawamoto K, Kobayashi S, et al. miR-146a suppresses the sensitivity to interferon-alpha in hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2011;414:675-680.
10. Borel F, Han R, Visser A, Petry H, van Deventer SJ, Jansen PL, et al. Adenosine triphosphate-binding cassette transporter genes up-regulation in untreated hepatocellular carcinoma is mediated by cellular microRNAs. *Hepatology* 2012;55:821-832.

Supporting Table 4. Circulating microRNAs as prognostic biomarkers for HCC

Experimental Setting	Material	Main finding	Reference
120 HCC, 135 HBV+, 48 HCV and 210 healthy controls	serum	Combined miR-25, miR-375 and let-7f upregulation in HBV and HCC	Li <i>et al</i> ; 2010 (1)
10 HCC patients and 10 healthy controls	plasma	miR-92a decrease in HCC patients	Shigoka <i>et al</i> ; 2010 (2)
46 HCC patients and 20 healthy controls	serum	Upregulation of miR221 has prognostic value	Li <i>et al</i> ; 2011(3)
105 HCC, 107 CLD patients and 71 healthy controls	serum	Downregulation of miR-16 and miR-199a	Qu <i>et al</i> ; 2011(4)
101 HCC patients, 48 CHB and 89 healthy controls	serum	Upregulation of miR-21, miR-122 and miR-123 in HCC and CHB	Xu <i>et al</i> ; 2011(5)
10 HBV+ HCC patients and 10 healthy controls	serum	miR-122 upregulation in HCC and CHB	Qi <i>et al</i> ; 2011(6)
457 HCC, 169 CHB, 141 cirrhotic patients and 167 healthy controls	plasma	Identification of a plasma miR-panel* with diagnostic value	Zhou <i>et al</i> ; 2011(7)
57 HCC patients and 30 healthy controls	serum	Combined increase of miR-15b and miR-130b as a classifier for HCC detection	Liu <i>et al</i> ; 2012(8)
32 HCC HCV+, 74 HCV+ and 12 healthy controls	urine	miR-618/650 increase for early diagnosis	Abdalla <i>et al</i> ; 2012(9)

HCC: hepatocellular carcinoma; HBV: Hepatitis B Virus; HCV: Hepatitis C virus; CHB: Chronic hepatitis B;

*miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801

Reference List

1. Li LM, Hu ZB, Zhou ZX, Chen X, Liu FY, Zhang JF, et al. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res* 2010;70:9798-9807.
2. Shigoka M, Tsuchida A, Matsudo T, Nagakawa Y, Saito H, Suzuki Y, et al. Deregulation of miR-92a expression is implicated in hepatocellular carcinoma development. *Pathol Int* 2010;60:351-357.
3. 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;406:70-73.
4. Qu KZ, Zhang K, Li H, Afdhal NH, Albitar M. Circulating microRNAs as biomarkers for hepatocellular carcinoma. *J Clin Gastroenterol* 2011;45:355-360.
5. 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;50:136-142.
6. Qi P, Cheng SQ, Wang H, Li N, Chen YF, Gao CF. Serum microRNAs as biomarkers for hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. *PLoS ONE* 2011;6:e28486.
7. 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;29:4781-4788.
8. Liu AM, Yao TJ, Wang W, Wong KF, Lee NP, Fan ST, et al. Circulating miR-15b and miR-130b in serum as potential markers for detecting hepatocellular carcinoma: a retrospective cohort study. *BMJ Open* 2012;2:e000825.
9. Abdalla MA, Haj-Ahmad Y. Promising Candidate Urinary MicroRNA Biomarkers for the Early Detection of Hepatocellular Carcinoma among High-Risk Hepatitis C Virus Egyptian Patients. *J Cancer* 2012;3:19-31.