

**Table 5.** Apoptosis-related miRNAs aberrantly expressed in HCC

Expression	miRNA(s)	Target(s) related-expression	References
Down-regulated in HCC	Let-7c	(+) BCL-xL	Shimizu <i>et al.</i> (2010) <sup>145</sup>
	Let-7g	(anti-apoptosis)	
	miR-15b	(+) Bcl-w	Chung <i>et al.</i> (2010) <sup>157</sup>
		(anti-apoptosis, drug resistance <sup>b</sup> )	
	miR-29	(+) Bcl-2, (+) Mlc-1	Xiong <i>et al.</i> (2010) <sup>150</sup>
		(anti-apoptosis)	
	miR-101	(+) Mlc-1 (anti-apoptosis)	Su <i>et al.</i> (2009) <sup>146</sup>
	miR-122	(+) Bcl-w (anti-apoptosis)	Lin <i>et al.</i> (2008) <sup>147</sup>
	miR-199a-3p	(+) c-MET, (+) mTOR	Fornari <i>et al.</i> (2010) <sup>116</sup>
		(anti-apoptosis, drug resistance <sup>b</sup> )	
Over-expressed in HCC	miR-223	(+) Stathmin 1 (anti-apoptosis)	Wong <i>et al.</i> (2008) <sup>70</sup>
	miR-15b	(-) BCL-w (anti-apoptosis)	Chung <i>et al.</i> (2010) <sup>157</sup>
	miR-21	(-) PTEN (tumor suppressor)	Meng <i>et al.</i> (2007) <sup>68</sup>
	miR-25	(-) Bim (pro-apoptosis)	Li <i>et al.</i> (2009) <sup>151</sup>
	miR-183	(-) PDCD4 (pro-apoptosis)	Li <i>et al.</i> (2010) <sup>156</sup>
	miR-221	(-) Bmf (pro-apoptosis)	Gramantieri <i>et al.</i> (2009) <sup>152</sup>
	miR-221	(-) PTEN (tumor suppressor)	Garofalo <i>et al.</i> (2009) <sup>124</sup>
	miR-222	Induces drug resistance <sup>b</sup>	
	miR-224	(-) API-5 (anti-apoptosis)	Wang <i>et al.</i> (2008) <sup>154</sup>
	miR-602	(-) RASSF1A (tumor suppressor, pro-apoptosis)	Yang <i>et al.</i> (2010) <sup>58</sup>

<sup>a</sup> TRAIL-induced apoptosis resistance; <sup>b</sup> drug resistance to doxorubicin-induced apoptosis

more aggressive phenotype with tumor dispersion and a reduced time to recurrence after surgery. *In vitro*, the enforced expression of miR-221 causes Bmf down-regulation, whereas miR-221 silencing induces its up-regulation and leads to an increase of apoptotic cell death.

MicroRNAs can also regulate programmed cell death by targeting other apoptosis-related genes. The most studied case is certainly miR-21, which is widely over-expressed in HCC tumors and cell lines (68). As miR-21 targets the tumor suppressor PTEN, its high expression in HCC may contribute to the promotion of HCC growth and spread. Moreover, the loss of PI3K pathway inhibition by PTEN is associated with major augmentation of cancer cell survival, leading to increased chemotherapy drug resistance. The abnormal and sustained activation of the PI3K pathway can also contribute to significant radio-resistance of hepatic tumors. Importantly, Tonimaru *et al.* demonstrated that miR-21 over-expression is responsible for the drug resistance of HCC cells to the chemotherapeutic combination interferon-alpha/5-fluorouracil (IFN-alpha/5-FU). Indeed, the transfection of HCC cell lines with miR-21 precursors increases IFN-alpha/5-FU resistance, whereas the use of anti-miR-21 renders the cells sensitive to the treatment (153). In addition, the limited expression level of miR-21 in human HCC tissue specimens was significantly associated with a favorable response to the IFN-alpha/5-FU combination therapy and the survival rate of the patients. Wang and coworkers analyzed the expression profiles of 157 miRNAs from 19 HCC patients and found that 22 miRNAs have abnormal up/down-regulated expression (154). Among them, miR-224 was the most significantly up-regulated miRNA. The authors demonstrated that miR-224 increases cell proliferation and sensitizes cell to apoptosis by targeting the apoptosis inhibitor-5 (API-5). Thus, the expression of miR-224 was found to be inversely correlated with the expression of API-5 in HCC patients. In their article, Yang and colleagues showed that miR-602 expression is aberrantly increased in chronic HBV-related hepatitis, cirrhosis, and HCC relative to the normal liver

(38). In addition, the specific expression of the miR-602 target, the tumor suppressor RASSF1A, was significantly decreased in the same samples. To finish, the inhibition of miR-602 in hepatoma cell lines *in vitro* leads to an increase of RASSF1A, promotes cell apoptosis, and inhibits cell proliferation. In another study, miR-223 has been shown to be down-regulated in HCC cell lines regardless of associated viral infection (70). The use of miR-223 precursors in HBV, HCV, and non-HBV/non-HCV cell lines revealed a consistent inhibitory effect on cell viability. The tumor suppressor effect of miR-223 probably involves the STMN1, one of its downstream targets. As previously reported STMN1 is a key microtubule-regulatory protein that is known to be frequently over-expressed in HCC, promoting cell viability and proliferation as well as decreasing apoptosis of HCC cells.

Numerous studies have already reported the dramatic role of aberrantly expressed miRNAs in the apoptosis of HCC cells as well as in drug resistance (124,127,155), as shown in Table 5. Among the most significant reports, miR-183 was identified as frequently up-regulated in HCC tissue samples and targeting programmed cell death 4 (PDCD4), a pro-apoptotic molecule involved in the TGF-beta1-induced apoptosis (156). Therefore, miR-183 is believed to play a major role in HCC development by significantly repressing PDCD4 expression and enhancing apoptosis inhibition. Fornari and colleagues showed that miR-199a-3p repression observed in HCC leads to the over-expression of mTOR and c-MET, two predictive targets of this miRNA. Conversely, the restoration of miR-199a-3p reduces the growth and invasive properties of HCC cell, whereas sensitivity to doxorubicin-induced apoptosis is increased (116). In addition, the analysis of human HCC tissues revealed an inverse correlation linking miR-199a-3p and mTOR, as well as shorter time of recurrence after HCC resection in the patients with lower miR-199a-3p. As a matter of fact, it is now undeniable that the expression of cancer-related miRNAs may predict the risk of tumor recurrence following curative resection. In particular, the expression of

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miR-15b in HCC cells has been reported to be negatively correlated with the reappearance of the disease (157). Thus, the high expression of miR-15b predicts a low risk of tumor recurrence following surgical resection. Experimentally, targeting miR-15b with antagonists increases HCC cell proliferation and inhibits the TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis *in vitro*, while miR-15b precursor transfection decreases proliferation and enhances apoptosis by repressing the anti-apoptotic Bcl-w. Consequently, the expression profiling of apoptosis-associated miRNAs may help to predict the drug resistance level of HCC tumors in patients. Besides their prognostic significance, modulating the expression of these specific miRNAs may represent a valuable method in order to promote apoptosis-sensitizing strategies for HCC treatment.

### 5. MIRNA PROFILING FOR THE CLINICAL DIAGNOSIS AND PROGNOSIS OF HCC: A REASONABLE PERSPECTIVE?

One of the objectives regarding the global-scale studies carried out in hepatic cancer was to identify the molecular actors contributing to the progression of the HCC from normal liver. The variability observed between patients regarding the prognosis of the disease shows that the HCC comprises several distinct biological phenotypes resulting from the activation of different oncogenic pathways during the tumorigenesis process. Consequently, the molecular profiling of miRNAs, as well as genes, proteins, and other molecules, appeared essential to identify the factors participating in hepatocarcinogenesis with the goal to develop accurate biomarkers for the diagnosis and precise staging of HCC. The accumulated evidence of the specific and frequent deregulations of miRNA expression in chronic hepatitis and liver cancer suggests their potential as promising biomarkers for these pathologies. To date, more than 500 articles related to microRNA and liver have been published since 2002. Among them, several miRNAs are reported to be significantly and differently expressed between HCCs and non-tumor adjacent tissues as well as between different HCC subtypes, highlighting the diagnostic and prognostic relevance of miRNA profiling (66,158).

In this context, the consideration of particular miRNA expression patterns associated with distinct pathological parameters could become useful for the clinical classification and prognostic stratification of the HCC. Presently, the tumor advancement (number/size of nodules, presence of vascular invasion, and extra-hepatic spread), the alteration of the hepatic functions, and the presence of portal hypertension are considered for the prognostic purpose and choice of treatment. Because the HCC is heterogeneous from both molecular and clinical perspectives, the available assessment methods of liver cancer could be improved and redefined by the incorporation of molecular data. The fact that each type of tumor is now believed to be associated with a specific profile of miRNA argues that such small RNAs could represent a valuable source of molecular diagnostic markers in order to discriminate distinct forms of cancerous lesions (8,74). Regarding liver cancer, the presence of

CSCs frequently contributes to HCC relapse due to the strong potential of these cells to metastasize as well as their resistance to cancer therapies. The miR-181 family has been reported to be up-regulated in HpSC-HCC (72) and may represent useful markers to identify HCC cases with a cancer stem cell feature, generally associated with poor prognosis. The c-MET expression signature also characterizes a subset of human HCCs associated with poor prognostic value and aggressive phenotype.

Budhu *et al.* defined 20 miRNAs as an HCC metastasis signature and showed that this 20 miRNA-based profile was capable of predicting survival and the recurrence of HCC in patients with multi-nodular or single tumors, including those at an early stage of the disease (159). Remarkably, the highlighted expression profile appeared to be a significant predictor of patient prognosis when compared to other clinical parameters, suggesting the clinical relevance of this miRNA signature. Furthermore, Li and co-workers demonstrated that a high expression of miR-125b is correlated with a good survival of HCC patients and can provide significance for the prognosis of liver cancer (160). Thus, profiling of aberrantly expressed cancer-related miRNAs might establish the basis for the development of a more rational system of classification in HCC diagnosis. It is plausible that miRNA signatures could help in the understanding of the advancement of liver cancer, allowing the consideration of more appropriate therapeutic strategies and a significant amelioration of patient survival.

Using miRNA profiling, several groups emphasized a plethora of miRNA signatures that might enable the differentiation of cancerous HCC tissues and normal liver tissues. Notably, each study highlighted a unique profile frequently lacking any miRNAs in common, which might be due to the difference of patient ethnicity, gender, underlying cause of liver disease, standardization method, control samples used (adjacent non-tumor tissue or hepatic samples from healthy donors), and/or techniques used for the screening. The fact that miRNA expression is heterogeneous between the studies also indicates that different miRNAs might be associated with unique tumor biology and distinct HCC outcomes. Several explanations argued for the existence of specific miRNA profiles reflecting the heterogeneity of the primitive liver cancer. As an example, HBV and HCV are both etiological agents for the development of the HCC that can alter miRNA expression. As shown previously, several miRNAs are clearly differentially expressed in the patients developing HCC with chronic HCV or HBV infection. Interestingly, the deregulation of most of these miRNAs is also associated in both cases, indicating the dual roles of miRNAs in viral replication and HCC development.

In addition to their potential implication in HCC diagnosis, the unique miRNA expression profiles previously reported and their correlation related to the advancement of the disease might also open the way to the development of novel curative strategies. Recent evidence indicates that the use of miRNA precursors or antagomiR might convincingly contribute to the development of

promising miRNA-based therapeutic compounds. As an example, Kota and colleagues showed that a systemic administration of miR-26a in mice inhibits the proliferation of HCC cells, leading to a remarkable slow-down of tumor progression without toxicity (108). These findings suggest that delivery of tumor suppressor type miRNAs, which are highly expressed and, therefore, tolerated in normal tissues but lost in transformed cells, may provide a general strategy for miRNA replacement therapies. In addition, the over-expression of miR-34 may also have a significant therapeutic value in both HCC and cholangiocarcinoma as miR-34 has been reported to target the anti-apoptotic protein family Bcl-2 and the receptor tyrosine kinase c-Met, two major oncogenes over-expressed in the HCC (137,161). In the drug-resistant EpCAM-positive HpSC-HCC, miR-181 conversely represents an attractive target, as the inhibition of this family of miRNAs significantly reduces the tumor potential of hepatic CSCs (72). The authors of this study hypothesized that combining chemotherapy and anti-miR-181 could be an interesting alternative of treatment for the resistant form of HpSC-HCC.

The primary liver cancer is a complex disease with a large variety of etiologic factors leading to multiple underlying tumorigenesis mechanisms. The lack of good molecular markers for the early diagnosis and the rational treatment of HCC represent a major challenge in clinical hepatology. However, the observations reviewed here give rise to significant hope that cancer-related miRNA expression signatures could serve in the establishment of a more relevant HCC grading system and provide new tools in order to improve the assessment of the lesion. Finally, developing non-invasive biomarkers for the diagnosis and the prognosis of liver cancer by using circulating miRNA profiling will certainly open the way to new possibilities in the management of HCC patients.

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## 7. REFERENCES

1. Farazi, P. A. & R. A. DePinho: Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat Rev Cancer*, 6, 674-87 (2006)
2. El-Serag, H. B. & K. L. Rudolph: Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*, 132, 2557-76 (2007)
3. Aravalli, R. N., C. J. Steer & E. N. Cressman: Molecular mechanisms of hepatocellular carcinoma. *Hepatology*, 48, 2047-63 (2008)
4. Bartel, D. P.: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116, 281-97 (2004)
5. Lewis, B. P., C. B. Burge & D. P. Bartel: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, 120, 15-20 (2005)
6. Lee, R. C., R. L. Feinbaum & V. Ambros: The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*, 75, 843-54 (1993)
7. Wightman, B., I. Ha & G. Ruvkun: Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell*, 75, 855-62 (1993)
8. Calin, G. A. & C. M. Croce: MicroRNA signatures in human cancers. *Nat Rev Cancer*, 6, 857-66 (2006)
9. Kent, O. A. & J. T. Mendell: A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. *Oncogene*, 25, 6188-96 (2006)
10. Calin, G. A., C. Seignani, C. D. Dumitru, T. Hyslop, E. Noch, S. Yendamuri, M. Shimizu, S. Rattan, F. Bullrich, M. Negrini & C. M. Croce: Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A*, 101, 2999-3004 (2004)
11. Esquela-Kerscher, A. & F. J. Slack: Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer*, 6, 259-69 (2006)
12. Calin, G. A., C. D. Dumitru, M. Shimizu, R. Bichi, S. Zupo, E. Noch, H. Aldler, S. Rattan, M. Keating, K. Rai, L. Rassenti, T. Kipps, M. Negrini, F. Bullrich & C. M. Croce: Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A*, 99, 15524-9 (2002)
13. Calin, G. A., M. Ferracin, A. Cimmino, G. Di Leva, M. Shimizu, S. E. Wojcik, M. V. Iorio, R. Visone, N. I. Sever, M. Fabbri, R. Iuliano, T. Palumbo, F. Pichiorri, C. Roldo, R. Garzon, C. Seignani, L. Rassenti, H. Alder, S. Volinia, C. G. Liu, T. J. Kipps, M. Negrini & C. M. Croce: A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med*, 353, 1793-801 (2005)
14. Cimmino, A., G. A. Calin, M. Fabbri, M. V. Iorio, M. Ferracin, M. Shimizu, S. E. Wojcik, R. I. Aqeilan, S. Zupo, M. Dono, L. Rassenti, H. Alder, S. Volinia, C. G. Liu, T. J. Kipps, M. Negrini & C. M. Croce: miR-15 and miR-16

## miRNAs in HCC diagnosis and prognosis

induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A*, 102, 13944-9 (2005)

15. Lu, J., G. Getz, E. A. Miska, E. Alvarez-Saavedra, J. Lamb, D. Peck, A. Sweet-Cordero, B. L. Ebert, R. H. Mak, A. A. Ferrando, J. R. Downing, T. Jacks, H. R. Horvitz & T. R. Golub: MicroRNA expression profiles classify human cancers. *Nature*, 435, 834-8 (2005)

16. Calin, G. A. & C. M. Croce: Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications. *J Clin Invest*, 117, 2059-66 (2007)

17. Volinia, S., G. A. Calin, C. G. Liu, S. Ambs, A. Cimmino, F. Petrocca, R. Visone, M. Iorio, C. Roldo, M. Ferracin, R. L. Prueitt, N. Yanaihara, G. Lanza, A. Scarpa, A. Vecchione, M. Negrini, C. C. Harris & C. M. Croce: A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A*, 103, 2257-61 (2006)

18. Meng, F., R. Henson, M. Lang, H. Wehbe, S. Maheshwari, J. T. Mendell, J. Jiang, T. D. Schmittgen & T. Patel: Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology*, 130, 2113-29 (2006)

19. Chan, J. A., A. M. Krichevsky & K. S. Kosik: MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res*, 65, 6029-33 (2005)

20. Liu, C. G., G. A. Calin, B. Meloon, N. Gamliel, C. Sevignani, M. Ferracin, C. D. Dumitru, M. Shimizu, S. Zupo, M. Dono, H. Alder, F. Bullrich, M. Negrini & C. M. Croce: An oligonucleotide microchip for genome-wide microRNA profiling in human and mouse tissues. *Proc Natl Acad Sci U S A*, 101, 9740-4 (2004)

21. Nelson, P. T., D. A. Baldwin, L. M. Scearce, J. C. Oberholtzer, J. W. Tobias & Z. Mourelatos: Microarray-based, high-throughput gene expression profiling of microRNAs. *Nat Methods*, 1, 155-61 (2004)

22. Hussain, S. M., P. E. Zondervan, I. J. JN, S. W. Schalm, R. A. de Man & G. P. Krestin: Benign versus malignant hepatic nodules: MR imaging findings with pathologic correlation. *Radiographics*, 22, 1023-36; discussion 1037-9 (2002)

23. Sakamoto, M., S. Hirohashi & Y. Shimosato: Early stages of multistep hepatocarcinogenesis: adenomatous hyperplasia and early hepatocellular carcinoma. *Hum Pathol*, 22, 172-8 (1991)

24. Choi, B. I., K. Takayasu & M. C. Han: Small hepatocellular carcinomas and associated nodular lesions of the liver: pathology, pathogenesis, and imaging findings. *AJR Am J Roentgenol*, 160, 1177-87 (1993)

25. Leong, T. Y. & A. S. Leong: Epidemiology and carcinogenesis of hepatocellular carcinoma. *HPB (Oxford)*, 7, 5-15 (2005)

26. Perz, J. F., G. L. Armstrong, L. A. Farrington, Y. J. Hutin & B. P. Bell: The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol*, 45, 529-38 (2006)

27. Saar, B. & F. Kellner-Weldon: Radiological diagnosis of hepatocellular carcinoma. *Liver Int*, 28, 189-99 (2008)

28. Befeler, A. S. & A. M. Di Bisceglie: Hepatocellular carcinoma: diagnosis and treatment. *Gastroenterology*, 122, 1609-19 (2002)

29. de Ledinghen, V., D. Laharie, R. Lecesne, B. Le Bail, M. Winnock, P. H. Bernard, J. Saric, P. Couzigou, C. Balabaud, P. Bioulac-Sage & J. Drouillard: Detection of nodules in liver cirrhosis: spiral computed tomography or magnetic resonance imaging? A prospective study of 88 nodules in 34 patients. *Eur J Gastroenterol Hepatol*, 14, 159-65 (2002)

30. Tong, M. J., L. M. Blatt & V. W. Kao: Surveillance for hepatocellular carcinoma in patients with chronic viral hepatitis in the United States of America. *J Gastroenterol Hepatol*, 16, 553-9 (2001)

31. Gupta, S., S. Bent & J. Kohlwes: Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med*, 139, 46-50 (2003)

32. Ryder, S. D.: Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. *Gut*, 52 Suppl 3, iii1-8 (2003)

33. Stewart, C. J., J. Coldewey & I. S. Stewart: Comparison of fine needle aspiration cytology and needle core biopsy in the diagnosis of radiologically detected abdominal lesions. *J Clin Pathol*, 55, 93-7 (2002)

34. Hoofnagle, J. H.: Course and outcome of hepatitis C. *Hepatology*, 36, S21-9 (2002)

35. Kiyosawa, K., T. Sodeyama, E. Tanaka, Y. Gibo, K. Yoshizawa, Y. Nakano, S. Furuta, Y. Akahane, K. Nishioka, R. H. Purcell & *et al.*: Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology*, 12, 671-5 (1990)

36. Ura, S., M. Honda, T. Yamashita, T. Ueda, H. Takatori, R. Nishino, H. Sunakozaka, Y. Sakai, K. Horimoto & S. Kaneko: Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology*, 49, 1098-112 (2009)

37. Zhang, X., S. Liu, T. Hu, S. Liu, Y. He & S. Sun: Up-regulated microRNA-143 transcribed by nuclear factor kappa B enhances hepatocarcinoma metastasis by repressing fibronectin expression. *Hepatology*, 50, 490-9 (2009)

38. Yang, L., Z. Ma, D. Wang, W. Zhao, L. Chen & G. Wang: MicroRNA-602 regulating tumor suppressive gene

## miRNAs in HCC diagnosis and prognosis

- RASSF1A is overexpressed in hepatitis B virus-infected liver and hepatocellular carcinoma. *Cancer Biol Ther*, 9, 803-8 (2010)
39. Liu, Y., J. J. Zhao, C. M. Wang, M. Y. Li, P. Han, L. Wang, Y. Q. Cheng, F. Zoulim, X. Ma & D. P. Xu: Altered expression profiles of microRNAs in a stable hepatitis B virus-expressing cell line. *Chin Med J (Engl)*, 122, 10-4 (2009)
40. Peng, X., Y. Li, K. A. Walters, E. R. Rosenzweig, S. L. Lederer, L. D. Aicher, S. Proll & M. G. Katze: Computational identification of hepatitis C virus associated microRNA-mRNA regulatory modules in human livers. *BMC Genomics*, 10, 373 (2009)
41. Marquez, R. T., S. Bandyopadhyay, E. B. Wendlandt, K. Keck, B. A. Hoffer, M. S. Icardi, R. N. Christensen, W. N. Schmidt & A. P. McCaffrey: Correlation between microRNA expression levels and clinical parameters associated with chronic hepatitis C viral infection in humans. *Lab Invest*, 90, 1727-36 (2010)
42. Morita, K., A. Taketomi, K. Shirabe, K. Umeda, H. Kayashima, M. Ninomiya, H. Uchiyama, Y. Soejima & Y. Maehara: Clinical significance and potential of hepatic microRNA-122 expression in hepatitis C. *Liver Int*, 31, 474-84 (2011)
43. Banaudha, K., M. Kaliszewski, T. Korolnek, L. Florea, M. L. Yeung, K. T. Jeang & A. Kumar: MicroRNA silencing of tumor suppressor DLC-1 promotes efficient hepatitis C virus replication in primary human hepatocytes. *Hepatology*, 53, 53-61 (2011)
44. Wanless, I. R. & J. S. Lentz: Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology*, 12, 1106-10 (1990)
45. Bacon, B. R., M. J. Farahvash, C. G. Janney & B. A. Neuschwander-Tetri: Nonalcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology*, 107, 1103-9 (1994)
46. Marchesini, G., M. Brizi, G. Bianchi, S. Tomassetti, E. Bugianesi, M. Lenzi, A. J. McCullough, S. Natale, G. Forlani & N. Melchionda: Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes*, 50, 1844-50 (2001)
47. Dolganiuc, A., J. Petrasek, K. Kodys, D. Catalano, P. Mandrekar, A. Velayudham & G. Szabo: MicroRNA expression profile in Lieber-DeCarli diet-induced alcoholic and methionine choline deficient diet-induced nonalcoholic steatohepatitis models in mice. *Alcohol Clin Exp Res*, 33, 1704-10 (2009)
48. Koteish, A. & A. Mae Diehl: Animal models of steatohepatitis. *Best Pract Res Clin Gastroenterol*, 16, 679-90 (2002)
49. Diehl, A. M.: Lessons from animal models of NASH. *Hepatol Res*, 33, 138-44 (2005)
50. Li, S., X. Chen, H. Zhang, X. Liang, Y. Xiang, C. Yu, K. Zen, Y. Li & C. Y. Zhang: Differential expression of microRNAs in mouse liver under aberrant energy metabolic status. *J Lipid Res*, 50, 1756-65 (2009)
51. Alisi, A., L. Da Sacco, G. Bruscalupi, F. Piemonte, N. Panera, R. De Vito, S. Leoni, G. F. Bottazzo, A. Masotti & V. Nobili: Mirnome analysis reveals novel molecular determinants in the pathogenesis of diet-induced nonalcoholic fatty liver disease. *Lab Invest*, 91, 283-93 (2011)
52. Wang, B., S. H. Hsu, S. Majumder, H. Kutay, W. Huang, S. T. Jacob & K. Ghoshal: TGFbeta-mediated upregulation of hepatic miR-181b promotes hepatocarcinogenesis by targeting TIMP3. *Oncogene*, 29, 1787-97 (2010)
53. Bala, S., M. Marcos, K. Kodys, T. Csak, D. Catalano, P. Mandrekar & G. Szabo: Up-regulation of microRNA-155 in macrophages contributes to increased tumor necrosis factor {alpha} (TNF{alpha}) production via increased mRNA half-life in alcoholic liver disease. *J Biol Chem*, 286, 1436-44 (2011)
54. Gines, P., A. Cardenas, V. Arroyo & J. Rodes: Management of cirrhosis and ascites. *N Engl J Med*, 350, 1646-54 (2004)
55. Bataller, R. & D. A. Brenner: Liver fibrosis. *J Clin Invest*, 115, 209-18 (2005)
56. Friedman, S. L.: Mechanisms of disease: Mechanisms of hepatic fibrosis and therapeutic implications. *Nat Clin Pract Gastroenterol Hepatol*, 1, 98-105 (2004)
57. Guo, C. J., Q. Pan, T. Cheng, B. Jiang, G. Y. Chen & D. G. Li: Changes in microRNAs associated with hepatic stellate cell activation status identify signaling pathways. *FEBS J*, 276, 5163-76 (2009)
58. Ji, J., J. Zhang, G. Huang, J. Qian, X. Wang & S. Mei: Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation. *FEBS Lett*, 583, 759-66 (2009)
59. Roderburg, C., G. W. Urban, K. Bettermann, M. Vucur, H. Zimmermann, S. Schmidt, J. Janssen, C. Koppe, P. Knolle, M. Castoldi, F. Tacke, C. Trautwein & T. Luedde: Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. *Hepatology*, 53, 209-18 (2011)
60. Murakami, Y., H. Toyoda, M. Tanaka, M. Kuroda, Y. Harada, F. Matsuda, A. Tajima, N. Kosaka, T. Ochiya & K. Shimotohno: The progression of liver fibrosis is related with overexpression of the miR-199 and 200 families. *PLoS One*, 6, e16081 (2011)
61. Gramantieri, L., F. Fornari, E. Callegari, S. Sabbioni, G. Lanza, C. M. Croce, L. Bolondi & M. Negrini: MicroRNA involvement in hepatocellular carcinoma. *J Cell Mol Med*, 12, 2189-204 (2008)

## miRNAs in HCC diagnosis and prognosis

62. Mott, J. L.: MicroRNAs involved in tumor suppressor and oncogene pathways: implications for hepatobiliary neoplasia. *Hepatology*, 50, 630-7 (2009)
63. Murakami, Y., T. Yasuda, K. Saigo, T. Urashima, H. Toyoda, T. Okanoue & K. Shimotohno: Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene*, 25, 2537-45 (2006)
64. Boyault, S., D. S. Rickman, A. de Reynies, C. Balabaud, S. Rebouissou, E. Jeannot, A. Hauriol, J. Saric, J. Belghiti, D. Franco, P. Bioulac-Sage, P. Laurent-Puig & J. Zucman-Rossi: Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology*, 45, 42-52 (2007)
65. Chen, X. M.: MicroRNA signatures in liver diseases. *World J Gastroenterol*, 15, 1665-72 (2009)
66. Ji, J. & X. W. Wang: New kids on the block: diagnostic and prognostic microRNAs in hepatocellular carcinoma. *Cancer Biol Ther*, 8, 1686-93 (2009)
67. Frankel, L. B., N. R. Christoffersen, A. Jacobsen, M. Lindow, A. Krogh & A. H. Lund: Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. *J Biol Chem*, 283, 1026-33 (2008)
68. Meng, F., R. Henson, H. Wehbe-Janek, K. Ghoshal, S. T. Jacob & T. Patel: MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology*, 133, 647-58 (2007)
69. Kutay, H., S. Bai, J. Datta, T. Motiwala, I. Pogribny, W. Frankel, S. T. Jacob & K. Ghoshal: Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. *J Cell Biochem*, 99, 671-8 (2006)
70. Wong, Q. W., R. W. Lung, P. T. Law, P. B. Lai, K. Y. Chan, K. F. To & N. Wong: MicroRNA-223 is commonly repressed in hepatocellular carcinoma and potentiates expression of Stathmin1. *Gastroenterology*, 135, 257-69 (2008)
71. Varnholt, H., U. Drebbler, F. Schulze, I. Wedemeyer, P. Schirmacher, H. P. Dienes & M. Odenthal: MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. *Hepatology*, 47, 1223-32 (2008)
72. Ji, J., T. Yamashita, A. Budhu, M. Forgues, H. L. Jia, C. Li, C. Deng, E. Wauthier, L. M. Reid, Q. H. Ye, L. X. Qin, W. Yang, H. Y. Wang, Z. Y. Tang, C. M. Croce & X. W. Wang: Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. *Hepatology*, 50, 472-80 (2009)
73. Yamashita, T., J. Ji, A. Budhu, M. Forgues, W. Yang, H. Y. Wang, H. Jia, Q. Ye, L. X. Qin, E. Wauthier, L. M. Reid, H. Minato, M. Honda, S. Kaneko, Z. Y. Tang & X. W. Wang: EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology*, 136, 1012-24 (2009)
74. Ladeiro, Y., G. Couchy, C. Balabaud, P. Bioulac-Sage, L. Pelletier, S. Rebouissou & J. Zucman-Rossi: MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. *Hepatology*, 47, 1955-63 (2008)
75. Datta, J., H. Kutay, M. W. Nasser, G. J. Nuovo, B. Wang, S. Majumder, C. G. Liu, S. Volinia, C. M. Croce, T. D. Schmittgen, K. Ghoshal & S. T. Jacob: Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. *Cancer Res*, 68, 5049-58 (2008)
76. Lujambio, A. & M. Esteller: CpG island hypermethylation of tumor suppressor microRNAs in human cancer. *Cell Cycle*, 6, 1455-9 (2007)
77. Girard, M., E. Jacquemin, A. Munnich, S. Lyonnet & A. Henrion-Caude: miR-122, a paradigm for the role of microRNAs in the liver. *J Hepatol*, 48, 648-56 (2008)
78. Krutzfeldt, J., N. Rajewsky, R. Braich, K. G. Rajeev, T. Tuschl, M. Manoharan & M. Stoffel: Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature*, 438, 685-9 (2005)
79. Czech, M. P.: MicroRNAs as therapeutic targets. *N Engl J Med*, 354, 1194-5 (2006)
80. Jopling, C. L., M. Yi, A. M. Lancaster, S. M. Lemon & P. Sarnow: Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science*, 309, 1577-81 (2005)
81. Tsai, W. C., P. W. Hsu, T. C. Lai, G. Y. Chau, C. W. Lin, C. M. Chen, C. D. Lin, Y. L. Liao, J. L. Wang, Y. P. Chau, M. T. Hsu, M. Hsiao, H. D. Huang & A. P. Tsou: MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. *Hepatology*, 49, 1571-82 (2009)
82. Gramantieri, L., M. Ferracin, F. Fornari, A. Veronese, S. Sabbioni, C. G. Liu, G. A. Calin, C. Giovannini, E. Ferrazzi, G. L. Grazi, C. M. Croce, L. Bolondi & M. Negrini: Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res*, 67, 6092-9 (2007)
83. Skotzko, M., L. Wu, W. F. Anderson, E. M. Gordon & F. L. Hall: Retroviral vector-mediated gene transfer of antisense cyclin G1 (CYCG1) inhibits proliferation of human osteogenic sarcoma cells. *Cancer Res*, 55, 5493-8 (1995)
84. Jensen, M. R., V. M. Factor, A. Fantozzi, K. Helin, C. G. Huh & S. S. Thorgeirsson: Reduced hepatic tumor incidence in cyclin G1-deficient mice. *Hepatology*, 37, 862-70 (2003)

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85. Kosaka, N., H. Iguchi & T. Ochiya: Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Sci*, 101, 2087-92 (2010)
86. Brase, J. C., D. Wuttig, R. Kuner & H. Sultmann: Serum microRNAs as non-invasive biomarkers for cancer. *Mol Cancer*, 9, 306 (2010)
87. Lawrie, C. H., S. Gal, H. M. Dunlop, B. Pushkaran, A. P. Liggins, K. Pulford, A. H. Banham, F. Pezzella, J. Boultonwood, J. S. Wainscoat, C. S. Hatton & A. L. Harris: Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol*, 141, 672-5 (2008)
88. Mitchell, P. S., R. K. Parkin, E. M. Kroh, B. R. Fritz, S. K. Wyman, E. L. Pogosova-Agadjanyan, A. Peterson, J. Noteboom, K. C. O'Briant, A. Allen, D. W. Lin, N. Urban, C. W. Drescher, B. S. Knudsen, D. L. Stirewalt, R. Gentleman, R. L. Vessella, P. S. Nelson, D. B. Martin & M. Tewari: Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A*, 105, 10513-8 (2008)
89. Ng, E. K., W. W. Chong, H. Jin, E. K. Lam, V. Y. Shin, J. Yu, T. C. Poon, S. S. Ng & J. J. Sung: Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut*, 58, 1375-81 (2009)
90. Chen, X., Y. Ba, L. Ma, X. Cai, Y. Yin, K. Wang, J. Guo, Y. Zhang, J. Chen, X. Guo, Q. Li, X. Li, W. Wang, Y. Zhang, J. Wang, X. Jiang, Y. Xiang, C. Xu, P. Zheng, J. Zhang, R. Li, H. Zhang, X. Shang, T. Gong, G. Ning, J. Wang, K. Zen, J. Zhang & C. Y. Zhang: Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res*, 18, 997-1006 (2008)
91. Wang, K., S. Zhang, B. Marzolf, P. Troisch, A. Brightman, Z. Hu, L. E. Hood & D. J. Galas: Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc Natl Acad Sci U S A*, 106, 4402-7 (2009)
92. Sukata, T., K. Sumida, M. Kushida, K. Ogata, K. Miyata, S. Yabushita & S. Uwagawa: Circulating microRNAs, possible indicators of progress of rat hepatocarcinogenesis from early stages. *Toxicol Lett*, 200, 46-52 (2011)
93. Zhang, Y., Y. Jia, R. Zheng, Y. Guo, Y. Wang, H. Guo, M. Fei & S. Sun: Plasma microRNA-122 as a biomarker for viral-, alcohol-, and chemical-related hepatic diseases. *Clin Chem*, 56, 1830-8 (2010)
94. Li, L. M., Z. B. Hu, Z. X. Zhou, X. Chen, F. Y. Liu, J. F. Zhang, H. B. Shen, C. Y. Zhang & K. Zen: Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res*, 70, 9798-807 (2010)
95. Qu, K. Z., K. Zhang, H. Li, N. H. Afdhal & M. Albitar: Circulating microRNAs as biomarkers for hepatocellular carcinoma. *J Clin Gastroenterol*, 45, 355-60 (2011)
96. Gui, J., Y. Tian, X. Wen, W. Zhang, P. Zhang, J. Gao, W. Run, L. Tian, X. Jia & Y. Gao: Serum microRNA characterization identifies miR-885-5p as a potential marker for detecting liver pathologies. *Clin Sci (Lond)*, 120, 183-93 (2011)
97. Xu, J., C. Wu, X. Che, L. Wang, D. Yu, T. Zhang, L. Huang, H. Li, W. Tan, C. Wang & D. Lin: Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. *Mol Carcinog*, 50, 136-42 (2011)
98. Li, J., Y. Wang, W. Yu, J. Chen & J. Luo: Expression of serum miR-221 in human hepatocellular carcinoma and its prognostic significance. *Biochem Biophys Res Commun*, 406, 70-3 (2011)
99. Yamamoto, Y., N. Kosaka, M. Tanaka, F. Koizumi, Y. Kanai, T. Mizutani, Y. Murakami, M. Kuroda, A. Miyajima, T. Kato & T. Ochiya: MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma. *Biomarkers*, 14, 529-38 (2009)
100. Shigoka, M., A. Tsuchida, T. Matsudo, Y. Nagakawa, H. Saito, Y. Suzuki, T. Aoki, Y. Murakami, H. Toyoda, T. Kumada, R. Bartenschlager, N. Kato, M. Ikeda, T. Takashina, M. Tanaka, R. Suzuki, K. Oikawa, M. Takanashi & M. Kuroda: Deregulation of miR-92a expression is implicated in hepatocellular carcinoma development. *Pathol Int*, 60, 351-7 (2010)
101. Braconi, C. & T. Patel: MicroRNA expression profiling: a molecular tool for defining the phenotype of hepatocellular tumors. *Hepatology*, 47, 1807-9 (2008)
102. Huang, S. & X. He: The role of microRNAs in liver cancer progression. *Br J Cancer*, 104, 235-40 (2011)
103. Villanueva, A., Y. Hoshida, S. Toffanin, A. Lachenmayer, C. Alsinet, R. Savic, H. Cornella & J. M. Llovet: New strategies in hepatocellular carcinoma: genomic prognostic markers. *Clin Cancer Res*, 16, 4688-94 (2010)
104. Bai, S., M. W. Nasser, B. Wang, S. H. Hsu, J. Datta, H. Kutay, A. Yadav, G. Nuovo, P. Kumar & K. Ghoshal: MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *J Biol Chem*, 284, 32015-27 (2009)
105. Zeng, C., R. Wang, D. Li, X. J. Lin, Q. K. Wei, Y. Yuan, Q. Wang, W. Chen & S. M. Zhuang: A novel GSK-3 beta-C/EBP alpha-miR-122-insulin-like growth factor 1 receptor regulatory circuitry in human hepatocellular carcinoma. *Hepatology*, 52, 1702-12 (2010)
106. Boutz, D. R., P. Collins, U. Suresh, M. Lu, C. M. Ramirez, C. Fernandez-Hernando, Y. Huang, R. de Sousa

- Abreu, S. Y. Le, B. A. Shapiro, A. M. Liu, J. M. Luk, S. F. Aldred, N. Trinklein, E. M. Marcotte & L. O. Penalva: A two-tiered approach identifies a network of cancer and liver diseases related genes regulated by miR-122. *J Biol Chem* (2011)
107. Xu, H., J. H. He, Z. D. Xiao, Q. Q. Zhang, Y. Q. Chen, H. Zhou & L. H. Qu: Liver-enriched transcription factors regulate microRNA-122 that targets CUTL1 during liver development. *Hepatology*, 52, 1431-42 (2010)
108. Kota, J., R. R. Chivukula, K. A. O'Donnell, E. A. Wentzel, C. L. Montgomery, H. W. Hwang, T. C. Chang, P. Vivekanandan, M. Torbenson, K. R. Clark, J. R. Mendell & J. T. Mendell: Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell*, 137, 1005-17 (2009)
109. Xu, T., Y. Zhu, Y. Xiong, Y. Y. Ge, J. P. Yun & S. M. Zhuang: MicroRNA-195 suppresses tumorigenicity and regulates G1/S transition of human hepatocellular carcinoma cells. *Hepatology*, 50, 113-21 (2009)
110. Xu, C., S. Liu, H. Fu, S. Li, Y. Tie, J. Zhu, R. Xing, Y. Jin, Z. Sun & X. Zheng: MicroRNA-193b regulates proliferation, migration and invasion in human hepatocellular carcinoma cells. *Eur J Cancer*, 46, 2828-36 (2010)
111. Imam, J. S., K. Buddavarapu, J. S. Lee-Chang, S. Ganapathy, C. Camosy, Y. Chen & M. K. Rao: MicroRNA-185 suppresses tumor growth and progression by targeting the Six1 oncogene in human cancers. *Oncogene*, 29, 4971-9 (2010)
112. Zhang, J., Y. Yang, T. Yang, Y. Liu, A. Li, S. Fu, M. Wu, Z. Pan & W. Zhou: microRNA-22, downregulated in hepatocellular carcinoma and correlated with prognosis, suppresses cell proliferation and tumourigenicity. *Br J Cancer*, 103, 1215-20 (2010)
113. Liu, A. M., R. T. Poon & J. M. Luk: MicroRNA-375 targets Hippo-signaling effector YAP in liver cancer and inhibits tumor properties. *Biochem Biophys Res Commun*, 394, 623-7 (2010)
114. Lan, F. F., H. Wang, Y. C. Chen, C. Y. Chan, S. S. Ng, K. Li, D. Xie, M. L. He, M. C. Lin & H. F. Kung: Hsa-let-7g inhibits proliferation of hepatocellular carcinoma cells by downregulation of c-Myc and upregulation of p16 (INK4A). *Int J Cancer*, 128, 319-31 (2011)
115. Cheng, J., L. Zhou, Q. F. Xie, H. Y. Xie, X. Y. Wei, F. Gao, C. Y. Xing, X. Xu, L. J. Li & S. S. Zheng: The impact of miR-34a on protein output in hepatocellular carcinoma HepG2 cells. *Proteomics*, 10, 1557-72 (2010)
116. Fornari, F., M. Milazzo, P. Chieco, M. Negrini, G. A. Calin, G. L. Grazi, D. Pollutri, C. M. Croce, L. Bolondi & L. Gramantieri: MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res*, 70, 5184-93 (2010)
117. Meng, X. Z., T. S. Zheng, X. Chen, J. B. Wang, W. H. Zhang, S. H. Pan, H. C. Jiang & L. X. Liu: microRNA expression alteration after arsenic trioxide treatment in HepG-2 cells. *J Gastroenterol Hepatol*, 26, 186-93 (2011)
118. Ivanovska, I., A. S. Ball, R. L. Diaz, J. F. Magnus, M. Kibukawa, J. M. Schelter, S. V. Kobayashi, L. Lim, J. Burchard, A. L. Jackson, P. S. Linsley & M. A. Cleary: MicroRNAs in the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. *Mol Cell Biol*, 28, 2167-74 (2008)
119. Liang, L., C. M. Wong, Q. Ying, D. N. Fan, S. Huang, J. Ding, J. Yao, M. Yan, J. Li, M. Yao, I. O. Ng & X. He: MicroRNA-125b suppressed human liver cancer cell proliferation and metastasis by directly targeting oncogene LIN28B2. *Hepatology*, 52, 1731-40 (2010)
120. Wu, N., X. Liu, X. Xu, X. Fan, M. Liu, X. Li, Q. Zhong & H. Tang: MiR-373, a novel regulator of PPP6C, functions as an oncogene in hepatocellular carcinoma. *Febs J* (2011)
121. le Sage, C., R. Nagel, D. A. Egan, M. Schrier, E. Mesman, A. Mangiola, C. Anile, G. Maira, N. Mercatelli, S. A. Ciafre, M. G. Farace & R. Agami: Regulation of the p27 (Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *Embo J*, 26, 3699-708 (2007)
122. Fornari, F., L. Gramantieri, M. Ferracin, A. Veronese, S. Sabbioni, G. A. Calin, G. L. Grazi, C. Giovannini, C. M. Croce, L. Bolondi & M. Negrini: MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. *Oncogene*, 27, 5651-61 (2008)
123. Pineau, P., S. Volinia, K. McJunkin, A. Marchio, C. Battiston, B. Terris, V. Mazzaferro, S. W. Lowe, C. M. Croce & A. Dejean: miR-221 overexpression contributes to liver tumorigenesis. *Proc Natl Acad Sci U S A*, 107, 264-9 (2010)
124. Garofalo, M., G. Di Leva, G. Romano, G. Nuovo, S. S. Suh, A. Ngankee, C. Taccioli, F. Pichiorri, H. Alder, P. Secchiero, P. Gasparini, A. Gonelli, S. Costinean, M. Acunzo, G. Condorelli & C. M. Croce: miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. *Cancer Cell*, 16, 498-509 (2009)
125. Liu, W. H., S. H. Yeh, C. C. Lu, S. L. Yu, H. Y. Chen, C. Y. Lin, D. S. Chen & P. J. Chen: MicroRNA-18a prevents estrogen receptor-alpha expression, promoting proliferation of hepatocellular carcinoma cells. *Gastroenterology*, 136, 683-93 (2009)
126. Ma, S., K. H. Tang, Y. P. Chan, T. K. Lee, P. S. Kwan, A. Castilho, I. Ng, K. Man, N. Wong, K. F. To, B. J. Zheng, P. B. Lai, C. M. Lo, K. W. Chan & X. Y. Guan: miR-130b Promotes CD133 (+) liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1. *Cell Stem Cell*, 7, 694-707 (2010)



127. Elyakim, E., E. Sitbon, A. Faerman, S. Tabak, E. Montia, L. Belanis, A. Dov, E. G. Marcusson, C. F. Bennett, A. Chajut, D. Cohen & N. Yerushalmi: hsa-miR-191 is a candidate oncogene target for hepatocellular carcinoma therapy. *Cancer Res*, 70, 8077-87 (2010)
128. Toffanin, S., Y. Hoshida, A. Lachenmayer, A. Villanueva, L. Cabellos, B. Minguez, R. Savic, S. C. Ward, S. Thung, D. Y. Chiang, C. Alsinet, V. Tovar, S. Roayaie, M. Schwartz, J. Bruix, S. Waxman, S. L. Friedman, T. Golub, V. Mazzaferro & J. M. Llovet: MicroRNA-Based Classification of Hepatocellular Carcinoma and Oncogenic Role of miR-517a. *Gastroenterology*, 140, 1618-1628 e16 (2011)
129. Yang, F., Y. Yin, F. Wang, Y. Wang, L. Zhang, Y. Tang & S. Sun: miR-17-5p Promotes migration of human hepatocellular carcinoma cells through the p38 mitogen-activated protein kinase-heat shock protein 27 pathway. *Hepatology*, 51, 1614-23 (2010)
130. Ding, J., S. Huang, S. Wu, Y. Zhao, L. Liang, M. Yan, C. Ge, J. Yao, T. Chen, D. Wan, H. Wang, J. Gu, M. Yao, J. Li, H. Tu & X. He: Gain of miR-151 on chromosome 8q24.3 facilitates tumour cell migration and spreading through downregulating RhoGDI. *Nat Cell Biol*, 12, 390-9 (2010)
131. Yao, J., L. Liang, S. Huang, J. Ding, N. Tan, Y. Zhao, M. Yan, C. Ge, Z. Zhang, T. Chen, D. Wan, M. Yao, J. Li, J. Gu & X. He: MicroRNA-30d promotes tumor invasion and metastasis by targeting Galphai2 in hepatocellular carcinoma. *Hepatology*, 51, 846-56 (2010)
132. Connolly, E. C., K. Van Doorslaer, L. E. Rogler & C. E. Rogler: Overexpression of miR-21 promotes an *in vitro* metastatic phenotype by targeting the tumor suppressor RHOB. *Mol Cancer Res*, 8, 691-700 (2010)
133. Wong, Q. W., A. K. Ching, A. W. Chan, K. W. Choy, K. F. To, P. B. Lai & N. Wong: MiR-222 overexpression confers cell migratory advantages in hepatocellular carcinoma through enhancing AKT signaling. *Clin Cancer Res*, 16, 867-75 (2010)
134. Yoon, S. O., S. M. Chun, E. H. Han, J. Choi, S. J. Jang, S. A. Koh, S. Hwang & E. Yu: Deregulated expression of microRNA-221 with the potential for prognostic biomarkers in surgically resected hepatocellular carcinoma. *Hum Pathol* (2011)
135. Kim, T., A. Veronese, F. Pichiorri, T. J. Lee, Y. J. Jeon, S. Volinia, P. Pineau, A. Marchio, J. Palatini, S. S. Suh, H. Alder, C. G. Liu, A. Dejean & C. M. Croce: p53 regulates epithelial-mesenchymal transition through microRNAs targeting ZEB1 and ZEB2. *J Exp Med*, 208, 875-83 (2011)
136. Kaposi-Novak, P., J. S. Lee, L. Gomez-Quiroz, C. Coulouarn, V. M. Factor & S. S. Thorgeirsson: Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. *J Clin Invest*, 116, 1582-95 (2006)
137. Li, N., H. Fu, Y. Tie, Z. Hu, W. Kong, Y. Wu & X. Zheng: miR-34a inhibits migration and invasion by down-regulation of c-Met expression in human hepatocellular carcinoma cells. *Cancer Lett*, 275, 44-53 (2009)
138. Salvi, A., C. Sabelli, S. Moncini, M. Venturin, B. Arici, P. Riva, N. Portolani, S. M. Giulini, G. De Petro & S. Barlati: MicroRNA-23b mediates urokinase and c-met downmodulation and a decreased migration of human hepatocellular carcinoma cells. *Febs J*, 276, 2966-82 (2009)
139. Wong, C. C., C. M. Wong, E. K. Tung, S. L. Au, J. M. Lee, R. T. Poon, K. Man & I. O. Ng: The microRNA miR-139 suppresses metastasis and progression of hepatocellular carcinoma by down-regulating Rho-kinase 2. *Gastroenterology*, 140, 322-31 (2011)
140. Wong, C. C., C. M. Wong, E. K. Tung, K. Man & I. O. Ng: Rho-kinase 2 is frequently overexpressed in hepatocellular carcinoma and involved in tumor invasion. *Hepatology*, 49, 1583-94 (2009)
141. Ji, J., L. Zhao, A. Budhu, M. Forgues, H. L. Jia, L. X. Qin, Q. H. Ye, J. Yu, X. Shi, Z. Y. Tang & X. W. Wang: Let-7g targets collagen type I alpha2 and inhibits cell migration in hepatocellular carcinoma. *J Hepatol*, 52, 690-7 (2010)
142. Bhattacharya, S. D., J. Garrison, H. Guo, Z. Mi, J. Markovic, V. M. Kim & P. C. Kuo: Micro-RNA-181a regulates osteopontin-dependent metastatic function in hepatocellular cancer cell lines. *Surgery*, 148, 291-7 (2010)
143. Shen, Q., V. R. Cicinnati, X. Zhang, S. Iacob, F. Weber, G. C. Sotiropoulos, A. Radtke, M. Lu, A. Paul, G. Gerken & S. Beckebaum: Role of microRNA-199a-5p and discoidin domain receptor 1 in human hepatocellular carcinoma invasion. *Mol Cancer*, 9, 227 (2010)
144. Wu, L., C. Cai, X. Wang, M. Liu, X. Li & H. Tang: MicroRNA-142-3p, a new regulator of RAC1, suppresses the migration and invasion of hepatocellular carcinoma cells. *FEBS Lett*, 585, 1322-30 (2011)
145. Shimizu, S., T. Takehara, H. Hikita, T. Kodama, T. Miyagi, A. Hosui, T. Tatsumi, H. Ishida, T. Noda, H. Nagano, Y. Doki, M. Mori & N. Hayashi: The let-7 family of microRNAs inhibits Bcl-xL expression and potentiates sorafenib-induced apoptosis in human hepatocellular carcinoma. *J Hepatol*, 52, 698-704 (2010)
146. Su, H., J. R. Yang, T. Xu, J. Huang, L. Xu, Y. Yuan & S. M. Zhuang: MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. *Cancer Res*, 69, 1135-42 (2009)
147. Lin, C. J., H. Y. Gong, H. C. Tseng, W. L. Wang & J. L. Wu: miR-122 targets an anti-apoptotic gene, Bcl-w, in

## miRNAs in HCC diagnosis and prognosis

human hepatocellular carcinoma cell lines. *Biochem Biophys Res Commun*, 375, 315-20 (2008)

148. Ma, L., J. Liu, J. Shen, L. Liu, J. Wu, W. Li, J. Luo, Q. Chen & C. Qian: Expression of miR-122 mediated by adenoviral vector induces apoptosis and cell cycle arrest of cancer cells. *Cancer Biol Ther*, 9, 554-61 (2010)

149. Fornari, F., L. Gramantieri, C. Giovannini, A. Veronese, M. Ferracin, S. Sabbioni, G. A. Calin, G. L. Grazi, C. M. Croce, S. Tavorari, P. Chieco, M. Negrini & L. Bolondi: MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res*, 69, 5761-7 (2009)

150. Xiong, Y., J. H. Fang, J. P. Yun, J. Yang, Y. Zhang, W. H. Jia & S. M. Zhuang: Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. *Hepatology*, 51, 836-45 (2010)

151. Li, Y., W. Tan, T. W. Neo, M. O. Aung, S. Wasser, S. G. Lim & T. M. Tan: Role of the miR-106b-25 microRNA cluster in hepatocellular carcinoma. *Cancer Sci*, 100, 1234-42 (2009)

152. Gramantieri, L., F. Fornari, M. Ferracin, A. Veronese, S. Sabbioni, G. A. Calin, G. L. Grazi, C. M. Croce, L. Bolondi & M. Negrini: MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality. *Clin Cancer Res*, 15, 5073-81 (2009)

153. Tomimaru, Y., H. Eguchi, H. Nagano, H. Wada, A. Tomokuni, S. Kobayashi, S. Marubashi, Y. Takeda, M. Tanemura, K. Umeshita, Y. Doki & M. Mori: MicroRNA-21 induces resistance to the anti-tumour effect of interferon-alpha/5-fluorouracil in hepatocellular carcinoma cells. *Br J Cancer*, 103, 1617-26 (2010)

154. Wang, Y., A. T. Lee, J. Z. Ma, J. Wang, J. Ren, Y. Yang, E. Tantoso, K. B. Li, L. L. Ooi, P. Tan & C. G. Lee: Profiling microRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-5 as a microRNA-224-specific target. *J Biol Chem*, 283, 13205-15 (2008)

155. Braconi, C., N. Valeri, P. Gasparini, N. Huang, C. Taccioli, G. Nuovo, T. Suzuki, C. M. Croce & T. Patel: Hepatitis C virus proteins modulate microRNA expression and chemosensitivity in malignant hepatocytes. *Clin Cancer Res*, 16, 957-66 (2010)

156. Li, J., H. Fu, C. Xu, Y. Tie, R. Xing, J. Zhu, Y. Qin, Z. Sun & X. Zheng: miR-183 inhibits TGF-beta1-induced apoptosis by downregulation of PDCD4 expression in human hepatocellular carcinoma cells. *BMC Cancer*, 10, 354 (2010)

157. Chung, G. E., J. H. Yoon, S. J. Myung, J. H. Lee, S. H. Lee, S. M. Lee, S. J. Kim, S. Y. Hwang, H. S. Lee & C. Y. Kim: High expression of microRNA-15b predicts a low risk of tumor recurrence following curative resection of hepatocellular carcinoma. *Oncol Rep*, 23, 113-9 (2010)

158. Bala, S., M. Marcos & G. Szabo: Emerging role of microRNAs in liver diseases. *World J Gastroenterol*, 15, 5633-40 (2009)

159. Budhu, A., H. L. Jia, M. Forgues, C. G. Liu, D. Goldstein, A. Lam, K. A. Zanetti, Q. H. Ye, L. X. Qin, C. M. Croce, Z. Y. Tang & X. W. Wang: Identification of metastasis-related microRNAs in hepatocellular carcinoma. *Hepatology*, 47, 897-907 (2008)

160. Li, W., L. Xie, X. He, J. Li, K. Tu, L. Wei, J. Wu, Y. Guo, X. Ma, P. Zhang, Z. Pan, X. Hu, Y. Zhao, H. Xie, G. Jiang, T. Chen, J. Wang, S. Zheng, J. Cheng, D. Wan, S. Yang, Y. Li & J. Gu: Diagnostic and prognostic implications of microRNAs in human hepatocellular carcinoma. *Int J Cancer*, 123, 1616-22 (2008)

161. He, L., X. He, L. P. Lim, E. de Stanchina, Z. Xuan, Y. Liang, W. Xue, L. Zender, J. Magnus, D. Ridzon, A. L. Jackson, P. S. Linsley, C. Chen, S. W. Lowe, M. A. Cleary & G. J. Hannon: A microRNA component of the p53 tumour suppressor network. *Nature*, 447, 1130-4 (2007)

162. Pogribny, I. P., A. Starlard-Davenport, V. P. Tryndyak, T. Han, S. A. Ross, I. Rusyn & F. A. Beland: Difference in expression of hepatic microRNAs miR-29c, miR-34a, miR-155, and miR-200b is associated with strain-specific susceptibility to dietary nonalcoholic steatohepatitis in mice. *Lab Invest*, 90, 1437-46 (2010)

163. Li, W. Q., C. Chen, M. D. Xu, J. Guo, Y. M. Li, Q. M. Xia, H. M. Liu, J. He, H. Y. Yu & L. Zhu: The rno-miR-34 family is upregulated and targets ACSL1 in dimethylnitrosamine-induced hepatic fibrosis in rats. *Febs J* (2011)

164. Huang, Y. S., Y. Dai, X. F. Yu, S. Y. Bao, Y. B. Yin, M. Tang & C. X. Hu: Microarray analysis of microRNA expression in hepatocellular carcinoma and non-tumorous tissues without viral hepatitis. *J Gastroenterol Hepatol*, 23, 87-94 (2008)

165. Jiang, J., Y. Gusev, I. Aderca, T. A. Mettler, D. M. Nagorney, D. J. Brackett, L. R. Roberts & T. D. Schmittgen: Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. *Clin Cancer Res*, 14, 419-27 (2008)

166. Connolly, E., M. Melegari, P. Landgraf, T. Tchaikovskaya, B. C. Tennant, B. L. Slagle, L. E. Rogler, M. Zavolan, T. Tuschl & C. E. Rogler: Elevated expression of the miR-17-92 polycistron and miR-21 in hepadnavirus-associated hepatocellular carcinoma contributes to the malignant phenotype. *Am J Pathol*, 173, 856-64 (2008)

167. Huang, X. H., Q. Wang, J. S. Chen, X. H. Fu, X. L. Chen, L. Z. Chen, W. Li, J. Bi, L. J. Zhang, Q. Fu, W. T. Zeng, L. Q. Cao, H. X. Tan & Q. Su: Bead-based microarray analysis of microRNA expression in hepatocellular carcinoma: miR-338 is downregulated. *Hepatol Res*, 39, 786-94 (2009)

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168. Ji, J., J. Shi, A. Budhu, Z. Yu, M. Forgues, S. Roessler, S. Ambs, Y. Chen, P. S. Meltzer, C. M. Croce, L. X. Qin, K. Man, C. M. Lo, J. Lee, I. O. Ng, J. Fan, Z. Y. Tang, H. C. Sun & X. W. Wang: MicroRNA expression, survival, and response to interferon in liver cancer. *N Engl J Med*, 361, 1437-47 (2009)

169. Furuta, M., K. I. Kozaki, S. Tanaka, S. Arii, I. Imoto & J. Inazawa: miR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. *Carcinogenesis*, 31, 766-76 (2009)

170. Liu, C., J. Yu, S. Yu, R. M. Lavker, L. Cai, W. Liu, K. Yang, X. He & S. Chen: MicroRNA-21 acts as an oncomir through multiple targets in human hepatocellular carcinoma. *J Hepatol*, 53, 98-107 (2010)

171. Huang, J., Y. Wang, Y. Guo & S. Sun: Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. *Hepatology*, 52, 60-70 (2010)

172. Hou, J., L. Lin, W. Zhou, Z. Wang, G. Ding, Q. Dong, L. Qin, X. Wu, Y. Zheng, Y. Yang, W. Tian, Q. Zhang, C. Wang, Q. Zhang, S. M. Zhuang, L. Zheng, A. Liang, W. Tao & X. Cao: Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell*, 19, 232-43 (2011)

**Abbreviations:** AFLD: alcohol fatty liver disease; AFP: alpha-fetoprotein; API-5: apoptosis inhibitor-5; CCL: cell lymphocytic leukemia; CCl<sub>4</sub>: carbon tetrachloride; CDAA: choline-deficient and amino acid; CSC: cancer stem cell; CT: computed tomography; Cyclin-CDK: cyclin-cyclin-dependent-kinase; EMT: epithelial to mesenchymal transition; DN: dysplastic nodules; EpCAM: epithelial cell adhesion molecule; FFPE: formalin-fixed paraffin-embedded; FNDC3B: fibronectin type III domain containing 3B; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HpSC-HCC: hepatic stem cell-like HCC; HSC: hepatic stellate cell; IFN-alpha: interferon-alpha; MCD: methionine-choline-deficient; MET, c-MET, or HGFR: hepatocyte growth factor receptor; miRNA: microRNA; MRI: magnetic resonance imaging; mTOR: mammalian target of rapamycin; MVB: multivesicular body; NAFLD: non-alcohol fatty liver disease; NF-kappaB: nuclear factor-kappa B; NT: non-tumor tissue; PDCD4: programmed cell death 4; PI3K: phosphatidylinositol tri-phosphate; pre-miRNA: precursor miRNA; pri-miRNA: primary miRNA; PTEN: phosphatase and tensin homolog; RISC: RNA-induced silencing complex; RT-qPCR: real-time polymerase chain reaction; siRNA: small interfering RNA; snRNA: small nucleolar RNA; STMN1: Stathmin 1; TGF-beta: transforming growth factor-beta; TRAIL: TNF-related apoptosis-inducing ligand; 3'-UTR: 3'-untranslated region; 5-FU: 5-fluorouracil

**Keys Words:** Hepatocellular Carcinoma, microRNA, Diagnosis, Prognosis, Review

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## Review

# Cancer-related microRNAs and their role as tumor suppressors and oncogenes in hepatocellular carcinoma

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**Summary.** MicroRNAs (miRNAs) have emerged as key factors involved in several biological processes, including development, differentiation, cell proliferation, and tumorigenesis. In hepatocellular carcinoma (HCC), miRNAs frequently present aberrant expression profiles, which make them potentially attractive for diagnostic or prognostic applications. Currently, accumulating evidence is indicating the role of miRNAs as tumor suppressors or oncogenes in hepatic malignancies. In particular, comprehensive studies have made possible a better understanding of HCC behavior, such as tumor growth, response to therapies, metastatic potential, or recurrence, regarding the altered expression of cancer-related miRNAs. Based on these findings, efforts are under way to define new markers for liver cancer in both invasive (hepatic biopsy or tumor resection) and non-invasive (circulating miRNAs in blood serum) ways. Due to their implication in the control of various cell processes altered in HCC, cancer-related miRNAs also offer encouraging perspectives for the development of innovative cancer therapies. In this article, we review the importance of miRNA deregulation in HCC progression and the role of these small non-coding RNAs as tumor suppressors and oncogenes. The significance of miRNAs in HCC diagnosis and miRNA-based therapeutic strategies is then discussed.

**Key words:** Hepatocellular carcinoma, MicroRNA, Tumor suppressor, Oncogene

## Introduction

Hepatocellular carcinoma (HCC) represents the third cause of death from cancer and the major form of liver malignancy worldwide, as it accounts for almost 90% of primitive hepatic tumors (Farazi and DePinho, 2006).

HCC is generally encountered in patients exhibiting an underlying chronic liver disease related to well-known risk factors, including hepatitis B virus (HBV) and/or C virus (HCV) infection, alcohol abuse, genetic diseases (e.g., hemochromatosis), genotoxic intoxication (e.g., aflatoxin B1), and liver steatosis. In the absence of diagnosis and clinical management, chronic hepatitis leads to fibrosis and gradually evolves into cirrhosis. Global studies estimate that approximately 80-90% of all HCCs arise from cirrhotic livers (El-Serag and Rudolph, 2007). To date, surgical resection and liver transplantation remain the only effective therapeutic options. However, a majority of the patients present an unresectable tumor due to late diagnosis, as such a disease tends to remain asymptomatic until late advancement or distant metastasis. In addition, chemotherapy resistance, high metastatic potential and tumor recurrence are generally associated with liver cancer, leading to a low survival rate (less than 10% after five years). Consequently, the discovery of innovative and effective biomarkers ensuring an early diagnosis of the disease in order to maximize the positive response of therapeutics before spreading and metastasizing remains an essential purpose in modern hepatology.

The critical role of microRNAs (miRNAs) has been described in the control of various biological processes frequently altered in cancer. In addition, several reports

**Abbreviations.** AFP: alpha-fetoprotein; CDK: cyclin-dependent kinase; CDKI: cyclin-dependent kinase inhibitor; DNMT: DNA methyltransferase; HBV: hepatitis B virus; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; IFN-alpha: interferon-alpha; MET, c-MET, or HGFR: hepatocyte growth factor receptor; miRNA: microRNA; MMP: matrix metalloprotease; mTOR: mammalian target of rapamycin; P-bodies: processing-bodies; pre-miRNA: precursor miRNA; pri-miRNA: primary miRNA; PTEN: phosphatase and tensin homolog; RISC: RNA-induced silencing complex; RT-qPCR: real-time quantitative polymerase chain reaction; TGF-beta: transforming growth factor-beta; TRAIL: TNF-related apoptosis-inducing ligand; 3'-UTR: 3'-untranslated region; 5-FU: 5-fluorouracil

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indicate that the altered expression of specific sets of miRNAs can contribute to liver tumorigenesis. Remarkably, even slight changes in the amount of a few miRNAs can substantially modify cellular physiology and contribute to carcinogenesis. It has been shown that more than 50% of miRNA genes are located at fragile sites or in cancer-associated genome regions (Calin et al., 2004). Therefore, following mutation, deletion, translocation, or amplification, miRNAs can be subjected to the same alterations as classic oncogenes or tumor suppressors (Esquela-Kerscher and Slack, 2006). In the last decade miRNA functions have begun to be elucidated, especially in the understanding of their major physiological implications. In mammals, miRNAs are predicted to participate in the regulation of almost all cellular processes, including development, cell differentiation, proliferation, and apoptosis (Bartel, 2009). As the abnormal expression of a number of miRNAs has been reported in a wide range of human cancers, a strong consensus has emerged that these cancer-related miRNAs may function as oncogenes or tumor suppressors (Calin and Croce, 2006; Kent and Mendell, 2006). Regardless of cell origin, a plethora of studies has revealed the overall and recurrent down-regulation of miRNAs in tumor tissues compared with normal tissues (Lu et al., 2005; Lujambio and Lowe, 2012). More recently, the establishment of miRNA signatures is interesting with regard to the management of liver cancer patients from both diagnostic and therapeutic perspectives (Gailhouste et al., 2013). In the present review, we describe the miRNA biogenesis mechanism and focus on miRNA-altered expression in liver cancer. By considering well-defined cases, the role of miRNAs as tumor suppressors and oncogenes is then explored. Lastly, the potential applications of cancer-related miRNAs for diagnosis and their therapeutic value in human HCC are discussed.

### MiRNA biogenesis and mechanism of action

MiRNAs are evolutionary conserved small non-coding RNAs of approximately 22 nucleotides that accurately regulate gene expression by complementary base pairing with the 3'-untranslated regions (3'-UTRs) of messenger RNAs (mRNAs) (Bartel, 2004). These fine post-transcriptional regulators were first evidenced in *C. elegans* by Ambros and co-workers, who discovered that *lin-4*, a gene known to control the timing of nematode larval development, did not code for a protein but produced small RNAs that can specifically bind to *lin-14* mRNA and repress its translation (Lee et al., 1993; Wightman et al., 1993). Then, miRNAs have been reported in a variety of organisms ranging from virus to mammals. In order to facilitate miRNA-based investigations, a miRNA registry (miRBase) has been established and is currently maintained by the University of Manchester (Griffiths-Jones, 2004). So far, 1921 mature miRNA sequences have been registered in the miRBase database (<http://www.mirbase.org>, release 18,

November 2011). It was estimated that more than 25% of all mature human miRNAs belong to a family comprising 2 or more members (based on a 7-seed sequence homology). As attested by computational studies, more than 30% of the protein-coding regions may be directly targeted and modulated by miRNAs (Lewis et al., 2005). An essential feature of miRNAs is that a single miRNA can recognize numerous mRNAs, and, conversely, one mRNA can be recognized by several miRNAs. These pleiotropic properties enable miRNAs to exert a wide control on a plethora of targets, attesting to the complexity of this mechanism of gene expression regulation. In addition, recent studies have demonstrated that certain miRNAs exhibit a tissue-specific distribution in rodent endoderm-derived tissues (Gao et al., 2011), although other miRNAs can be expressed ubiquitously (Lagos-Quintana et al., 2002). Thus, endodermal-derived hepatic and pancreatic tissues display differential miRNA enrichment in comparison to other organs. Typically, miR-122, miR-21, miR-101, miR-192, and miR-221 expression progressively increases during liver morphogenesis to become predominant in the adult hepatic tissue. Among these miRNAs highly expressed in hepatocytes, the liver-specific miR-122 represents 70% of the total amount of miRNAs in the organ. Intriguingly, the pri- and pre-miR-122 are regulated in a circadian manner (Gatfield et al., 2009). In addition, the turnover of mature miR-122 appears to be relatively long compared to other miRNAs, as its half-life may reach several weeks.

MiRNA biogenesis is a multistep process that has been reviewed extensively (Fig. 1). Briefly, miRNAs are produced by the RNA polymerase II into transcriptional precursors of hundreds of nucleotides called primary miRNAs (pri-miRNAs). These long primary precursor transcripts exhibit several stem-loop structures of approximately 80 nucleotides. In the nucleus, pri-miRNAs undergo processing by the nuclear endonuclease Drosha and the double-stranded RNA-binding protein Pasha to be cleaved into precursor miRNAs (pre-miRNAs). Pre-miRNAs are then exported to the cytoplasm by exportin-5, where they undergo further processing by the RNase III endonuclease Dicer. Dicer cleaves the pre-miRNA loop to produce an imperfect duplex consisting of a mature miRNA and a complementary fragment of a similar size (miRNAs\*). A mature miRNA measures 20 to 23 nucleotides in length, which can be incorporated into the RNA-induced silencing complex (RISC), whereas the complementary miRNA\* separates from the duplex and is generally degraded. Functional target sites within the mRNA usually consist of a 6-7-nucleotide-long sequence, the so-called miRNA "seed sequence." The silencing complex binds complementarily the 3'-UTR of the target sequences and negatively regulates gene expression either through the endonucleolytic cleavage of the mRNA or inhibition of its translation (Bartel, 2004). Lastly, miRNAs and their target mRNAs will be localized in the cytoplasmic processing-bodies (P-

bodies) where they will be degraded (Liu et al., 2005). The turnover of miRNAs is still a largely unexplored area. However, RNA degradation enzymes might target not only mature miRNAs but also the precursor pri- and pre-miRNAs. In humans, the decrease of mRNA levels related to miRNA activity has been shown to precede protein diminution in 84% of cases (Bartel, 2009).

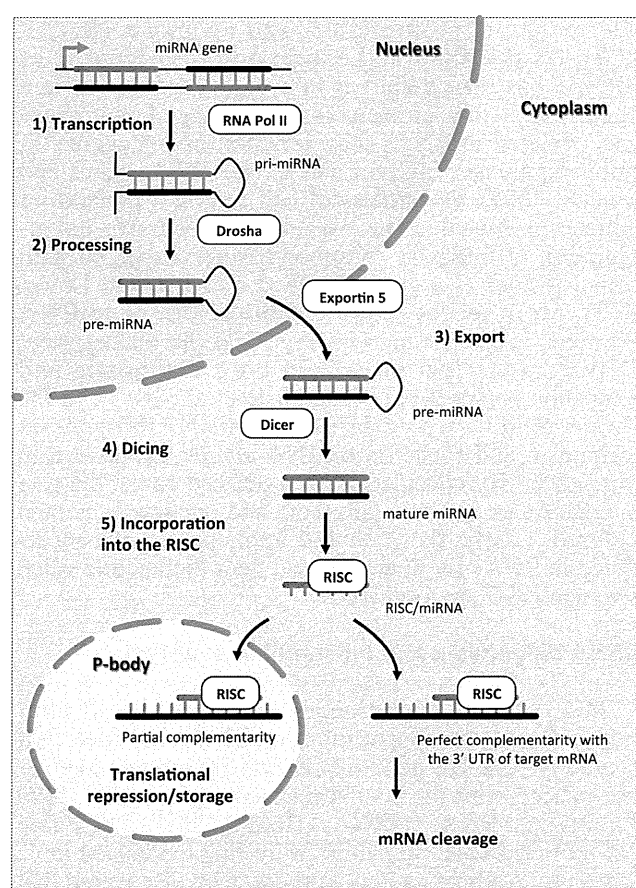
### Cancer-related miRNAs and their altered expression in HCC

MiRNA expression can be regulated at various levels from sequence identity, processing, stability and mRNA binding. Thus, all these steps are susceptible to be altered in cancer cells, impacting the global production of miRNAs. In general, miRNAs display a globally repressed expression profile regardless of tumor origin (Lu et al., 2005). However, the oncogenic properties of a number of miRNAs and their over-expression in several types of cancers have also been reported (Calin and Croce, 2007).

The progression of HCC generally involves various genetic and epigenetic aberrations (Aravalli et al., 2008). Genetic alterations usually result from chromosomal abnormalities that can lead to the depletion, amplification, or translocation of miRNAs. Approximately 50% of all annotated human miRNA genes are located at fragile sites of the genome that are associated with cancer (Calin et al., 2004). For instance, the gene that codes for miR-21 is located in a region at chromosome 17q23.2 frequently amplified in various types of solid tumors (Volinia et al., 2006). Epigenetic mechanisms and DNA methylation are also critical for miRNA regulation (Sato et al., 2011). Thus, it has been demonstrated that the down-regulation of several tumor suppressor miRNAs observed in cancer cells is mediated by the hyper-methylation of their promoting sequences (Lujambio and Esteller, 2007; Datta et al., 2008). For example, Furuta and colleagues showed that the silencing of the tumor-suppressive miR-124 and miR-203 through CpG-island methylation represents a major event in hepatocarcinogenesis (Furuta et al., 2010). This aberrant methylation is frequently due to the increased expression of DNA methyltransferase (DNMT) enzymes and observed in a number of human cancers, including HCC (Kondo et al., 2000). The case of miR-148a and miR-152 is of interest as the inactivation of these two miRNAs by DNA methylation frequently occurs in malignancies, such as gastric tumors (Zhu et al., 2011), pancreatic cancers (Hanoun et al., 2010), and cholangiocarcinoma (Braconi et al., 2010).

In addition to genomic and epigenetic alterations, miRNA processing itself is frequently altered in liver cancer. First, the transcription of pri-miRNAs can be regulated and modified by several transcription factors. The oncogenic transcription factor c-Myc binds, for instance, the upstream of let-7 and miR-26a, repressing the transcription of these two miRNAs and contributing to tumorigenesis (Chang et al., 2008). Another crucial

point is the altered expression of DICER. In most cancer types, the down-regulation of DICER has been shown to be related to the global deregulation of miRNA expression (Faggad et al., 2010; Wu et al., 2011a-c). In the liver, Sekine and co-workers tested the consequence of DICER1 depletion by performing conditional knockout in hepatocytes (Sekine et al., 2009). Remarkably, the hepatocytes exhibiting DICER1-specific depletion displayed a gene expression profile indicative of cell growth and de-differentiation into liver progenitors. At one year of age, approximately 60% of the mutant mice spontaneously developed HCC derived



**Fig. 1.** The RNA interference process: biogenesis and regulation of miRNAs. Transcription from the miRNA genes by the RNA polymerase II occurs in the nucleus. The primary precursor miRNAs (pri-miRNAs) are then cleaved by the RNase III enzyme Drosha, producing precursor molecules (pre-miRNAs). With the help of the Exportin-5, the pre-miRNAs are exported into the cytoplasm, where they undergo further processing by the ribonucleases Dicer to generate mature miRNAs. Mature single-stranded miRNAs are incorporated onto the RNA-induced silencing complex (RISC) to carry out their silencing function. The regulation mechanism is dependent on the degree of complementarity between the 3'-UTR region of the target mRNA and the seed region in the 5'-end of the miRNA. In the case of perfect complementarity, the mRNA is cleaved by RISC. If the complementarity is partial, the regulation is carried out by repression of the translation in the P-body.

from the DICER1-deficient hepatocytes. In another study, TARBP2 (TAR RNA-binding protein 2), encoding an integral component of a DICER1-containing complex, has been described as important for maintaining DICER1 stability, its mutation leading to DICER1 alteration and the global down-regulation of miRNAs (Melo et al., 2009). Lastly, and on the mRNA side, oncogenes acquire mutations that remove miRNA-binding sites in tumor cells. This phenomenon has been described in lipoma development, where the disruption of the pairing between let-7 and HMGA2 enhanced oncogenic transformation (Mayr et al., 2007).

### MiRNA as a diagnostic tool

In recent years, several groups have reported the over-expression or the down-regulation of a number of miRNAs in a large variety of cancers. MiRNA signatures are believed to serve as accurate molecular biomarkers for the clinical classification of tumors as well as for the development of innovative therapeutic strategies. Therefore, the availability of consistent technologies that enable the detection of miRNAs has become of interest for both fundamental and clinical purposes. The current detection methods commonly used are microarray, real-time quantitative polymerase chain reaction (RT-qPCR), next generation sequencing (NGS), and, in a less routine way, Northern blot or *in situ* hybridization. Microarray analyses present the advantage of offering a high speed of screening by employing various miRNA probes within a single microchip. However, the technique has lower sensitivity and specificity than RT-qPCR, which is still the gold standard for miRNA analyses. MiRNA RT-qPCR is based on the use of stem-loop primers, which can specifically bind to the mature miRNA during reverse transcription, conferring a high degree of accuracy to the method (Chen et al., 2005). Analysis of miRNAs by RT-qPCR is a cost-effective technique and, due to its efficiency, a valuable way for the validation of miRNA signatures. Moreover, the development of RT-qPCR protocols has improved the sensitivity of miRNA detection down to a few nanograms of total RNAs. This amount can be easily and routinely obtained by extracting total RNAs from a small fragment of a hepatic percutaneous biopsy. However, the procedures employed for the normalization of miRNA data still remain a major point of discordance that requires attention. Indeed, distinct miRNA signatures have been emphasized despite the fact that miRNA profiling assays were carried out under similar conditions (the same type of tumor and the same screening technique). This dispersion might be caused by variations in patient population, such as ethnicity, gender, difference in diagnostic classification, as well as the standardization methods employed or the references used (adjacent non-tumor tissue or hepatic samples from healthy donors). Obviously, the disparity observed between miRNA profiles established in the same type of cancer may also

reflect different underlying causes of the diseases as well as diverse HCC outcomes.

The aberrant expression of cancer-related miRNAs in HCC frequently contributes to the deregulation of a tumor suppressor and/or oncogene pathways, indicating the direct and crucial role of miRNAs in liver carcinogenesis (Gramantieri et al., 2008; Mott, 2009; Gailhouste et al., 2013). Different miRNA signatures have also been related to chronic hepatic infections (Calin and Croce, 2006), cirrhosis, and steatosis (Cheung et al., 2008). Using microarray technologies, Murakami and colleagues were one of the first groups to report a pattern of 7 mature miRNAs that exhibit differential expression between HCC and adjacent non-tumor samples (Murakami et al., 2006). In the 25 pairs analyzed, 5 miRNAs appeared to be significantly down-regulated (miR-199a, miR-199a\*, miR-195, miR-200a, and miR-125a), whereas 2 miRNAs displayed a higher expression in HCC samples (miR-18 and miR-224). Ladeiro and co-workers also identified specific miRNA expression profiles that can unambiguously differentiate between benign and malignant HCCs as well as between several subtypes of HCC tumors (Ladeiro et al., 2008). In this study, HCC tumors exhibited a redundant over-expression of miR-224 regardless of the underlying disease. Moreover, the case of the hepatospecific miR-122 may also be of prime interest. Krutzfeldt and colleagues demonstrated that the silencing of miR-122 by using antagomir resulted in an increased expression of hundreds of genes known to be putatively targeted by miR-122 and normally repressed in normal hepatocytes (Krutzfeldt et al., 2005). This argues for the involvement of miR-122 in maintaining the “adult-liver” phenotype by suppressing the expression of several non-hepatic genes. Furthermore, the replication of HCV is known to be related to the expression of miR-122 in infected cells. Thus, HCV viral RNA can replicate in the Huh-7 cell line, which expresses miR-122, but not in HepG2 cells, which do not express miR-122. In addition, the experimental sequestration of miR-122 in cells leads to a marked loss of HCV RNA replication (Jopling et al., 2005). Importantly, miR-122 was highlighted as down-regulated in more than 70% of the samples from HCC patients with underlying cirrhosis, as well as in 100% of the HCC-derived cell lines analyzed (Gramantieri et al., 2007). Finally, miR-122 knock-out mice display hepatosteatosis, fibrosis, and a high incidence of HCC, suggesting the tumor suppressor role of miR-122 in the liver (Hsu et al., 2012).

A plethora of studies have reported various miRNA profiles potentially reflecting HCC initiation and progression which could be employed as specific cancer biomarkers (Chen, 2009; Ji and Wang, 2009). To generalize, miR-21, miR-221, miR-222, and miR-224 were widely reported as up-regulated in HCC, whereas miR-122, miR-199, miR-223, and the let-7 family members were frequently found to be down-regulated in most studies. Considering these data, the establishment of accurate miRNA-based signatures could be of prime

interest in the development of new tools for the diagnosis and advancement staging of liver cancer. However, elucidating the functional implication of the hepatic cancer-related miRNAs and the consequence of their deregulation in HCC progression also remains of prime interest from the perspective of a miRNA-based curative management.

### Circulating miRNAs

To date, the development of consistent and reproducible methods to improve the diagnosis of HCC at an early stage in a non-invasive way represents a real necessity in clinical hepatology. HCC tissues secrete various tumor-related compounds into the blood, and these may serve as circulating biomarkers for the diagnosis of liver cancer. It was recently proposed that miRNAs can be conveyed in blood serum, participating in intercellular communication or conditioning the tumor environment (Kosaka et al., 2010, 2012). The concept that miRNAs could serve as potential plasma markers for liver diseases is, thus, gaining attention. Moreover, the American Association for the Study of Liver Diseases (AASLD), in its practice guideline, recently discontinued the use of the blood tumor marker alpha-fetoprotein (AFP) for surveillance and diagnosis, increasing the need for novel HCC biomarkers in blood tests.

Tumor-derived miRNAs have been efficiently detected in the serum of liver disease patients and characterized as potential biomarkers for HCC (Borel et al., 2012). In a relevant manner, the levels of 3 miRNAs (miR-21, miR-122, and miR-223) have been found to be significantly elevated in the serum of the patients exhibiting HBV infection or HBV-related HCC (Xu et al., 2011). A study carried out by Li and collaborators highlighted a specific set of miRNAs significantly up-regulated in HBV-positive HCC samples (Li et al., 2010a,b). Among them, miR-122 was highly increased in the serum of HBV patients but not in those of HCV patients. By employing a combination of characterized miRNAs, the authors could finally discriminate HCC cases from the controls or the infected non-HCC patients. Another study also emphasized the prognostic significance of serum miR-221, which represents another miRNA frequently over-expressed in HCC. In this report, the high expression of circulating miR-221 was correlated with the size of the tumor and the advancement of the disease (Li et al., 2011). Furthermore, the overall survival rate of patients exhibiting high levels of serum miR-221 was significantly lower than that of patients with low miR-221 rates. Remarkably, miR-500 has also been detected in increased amounts in the serum of HCC patients and found to be significantly reduced after the surgical resection of their tumor (Yamamoto et al. 2009). Conversely, Shigoka and collaborators highlighted the low level of circulating miR-92a in HCC, whereas tumor

resection was followed by the drastic augmentation of this miRNA in blood serum (Shigoka et al., 2010). More recently, Tomimaru and collaborators evaluated the significance of plasma miRNAs as biochemical markers for HCC and demonstrated the relevance of assessing circulating miR-21 for the non-invasive diagnosis of liver cancer (Tomimaru et al., 2012).

To validate the clinical relevance of serum miRNAs, further studies will be required, with a special focus on the standardization methods or the choice of the most appropriate miRNAs for internal references. Despite the availability of good endogenous normalizers in liver tissues, no circulating miRNAs have been identified and validated as a standard reference. Although the process of assessing serum miRNAs remains under improvement and will require procedures for validations, cancer-related circulating miRNAs represent an exciting and promising field of investigation in order to develop more accurate technologies for the non-invasive diagnosis of HCC.

### Tumor suppressive miRNAs and oncomirs

Under physiological conditions, the mechanisms for DNA repair, cell proliferation, motility, and programmed cell death are tightly regulated in order to maintain tissue homeostasis. Alteration of critical genes that modulate these cellular processes may tilt this balance, predisposing cells to transformation. MiRNAs have been closely associated with a number of these critical genes and found to exert an essential role in conditioning tumorigenesis and cancer progression. The current consensus is that cancer-related miRNAs function as oncogenes or tumor suppressors (Calin and Croce, 2006; Gailhouse et al., 2013). As for other malignancies, two situations can occur in HCC: i) tumor suppressor miRNAs can be down-regulated in HCC and cause the up-regulation of oncogenic target genes, repressed in normal hepatic tissues, increasing cell growth, migration, or invasion, and potentially leading to hepatocarcinogenesis (Table 1); ii) oncogenic miRNAs can be up-regulated in HCC and lead to the down-regulation of target tumor suppressor genes, participating in the development of the cancer phenotype (Table 2). Importantly, the expression profile of certain miRNAs has been found to reflect the biological behavior of HCC tumors, such as aggressiveness, invasiveness, or drug resistance. As a consequence, miRNA investigations may offer the opportunity to determine miRNA signatures that would provide valuable information to stratify and refine HCC diagnosis in terms of prognosis, response to treatment, and disease relapse. However, the gap between bench and bedside has not been bridged, and a better understanding of the cellular mechanisms that are altered in HCC through miRNA deregulation will be required to identify the miRNAs that would serve as relevant diagnostic makers and therapeutic targets for clinical practice.



**Table 1.** Key tumor suppressor miRNAs down-regulated in HCC.

Function	miRNAs	Target(s)	References
<b>Cell growth/Proliferation (-)</b>			
	Let-7g	c-Myc	Lan et al. (2011)
	miR-1	FoxP1, c-MET, HDAC4	Datta et al. (2008)
	miR-7	PIK3CD, mTOR, p70S6K	Fang et al. (2012)
	miR-22	HDAC4	Zhang et al. (2010)
	miR-26a	Cyclin D2, Cyclin E2	Kota et al. (2009)
	miR-29a	PPM1D	Meng et al. (2011)
	miR-34a	MACF1	Cheng et al. (2010)
	miR-99a	IGF-1R, mTOR	Li et al. (2011)
	miR-122	Cyclin G1 SRF IGF1R PTPN1, SEPT2, SEPT9 CUTL1	Gramantieri et al. (2007); Fornari et al. (2009) Bai et al. (2009) Bai et al. (2009); Zeng et al. (2010) Boutz et al. (2011) Xu et al. (2010)
	miR-124	CDK6	Furuta et al. (2010)
	miR-125b	Not determined	Liang et al. (2010)
	miR-185	Six1	Imam et al. (2010)
	miR-193b	Cyclin D1, ETS1	Xu et al. (2010)
	miR-195	Cyclin D1, E2F3, CDK6	Xu et al. (2009)
	miR-199a-3p	mTOR	Fornari et al. (2010)
	miR-219-5p	GPC3	Huang et al. (2012)
	miR-223	STMN1	Wong et al. (2008)
	miR-375	YAP AEG-1	Liu et al. (2010) He et al. (2012)
	miR-449	c-MET	Buurman et al. (2012)
	miR-519d	MKI67	Hou et al. (2011)
	miR-637	LIF	Zhang et al. (2011)
<b>Cell motility/Invasion (-)</b>			
	let-7g	COL1A2	Ji et al. (2010)
	miR-1	c-MET	Datta et al. (2008)
	miR-7	PIK3CD, mTOR, p70S6K	Fang et al. (2012)
	miR-23b	c-MET, uPA	Salvi et al. (2009)
	miR-34a	c-MET LMNA, GFAP, MACF1, ALDH2	Li et al. (2009) Cheng et al. (2010)
	miR-122	ADAM10 ADAM17 MMP7, PNX	Bai et al. (2009) Tsai et al. (2009) Boutz et al. (2011)
	miR-124	ROCK2, EZH2	Zheng et al. (2012)
	miR-125b	LIN28B2	Liang et al. (2010)
	miR-139	ROCK2	Wong et al. (2011)
	miR-142-3p	RAC1	Wu et al. (2011)
	miR-181a	OPN	Bhattacharya et al. (2010)
	miR-185	Six1	Imam et al. (2010)
	miR-193b	Cyclin D1, ETS1	Xu et al. (2010)
	miR-199a-3p	c-MET, mTOR	Fornari et al. (2010)
	miR-199a-5p	DDR1	Shen et al. (2010)
	miR-375	AEG-1	He et al. (2012)
<b>Cell viability (-)/apoptosis (+)</b>			
	Let-7c	Bcl-xL	Shimizu et al. (2010)
	Let-7g		
	miR-15b	Bcl-w	Chung et al. (2010)
	miR-29	Bcl-2, Mlc-1	Xiong et al. (2010)
	miR-101	Mlc-1	Su et al. (2009)
	miR-122	Bcl-w	Lin et al. (2008)
	miR-199a-3p	c-MET, mTOR	Fornari et al. (2010)
	miR-223	Stathmin 1	Wong et al. (2008)
	miR-375	AEG-1	He et al. (2012)
	miR-449	c-MET	Buurman et al. (2012)
	miR-637	LIF	Zhang et al. (2011)

## Tumor suppressor miRNAs

### Cell proliferation/tumor growth

In several studies, the role of specific miRNAs has been reported in the regulation of the proliferation signaling pathways by a direct interaction with critical cell cycle regulators. Among those miRNAs are tumor suppressors targeting cyclin-cyclin-dependent-kinase (Cyclin-CDK) complexes, a class of positive modulators

of the cell cycle. One of the miRNAs identified as a major regulator of cyclin-CDK complexes in liver cancer is miR-26a. MiR-26a has been shown to induce cell cycle arrest by directly targeting cyclin D2 and E2 (Kota et al., 2009). Moreover, miR-195 exerts a tumor suppressor activity by targeting CDK6 (Xu et al., 2009). This miRNA can also inhibit the G1/S transition by repressing cyclin D1. In addition to proving the overall inhibition of miR-122 in liver cancer, Gramantieri and colleagues also demonstrated the existence of an inverse

**Table 2.** Key oncomirs over-expressed in HCC.

Function	miRNAs	Target(s)	References
Cell growth/Proliferation (+)			
	miR-18a	ESR1	Liu et al. (2009)
	miR-21	PTEN	Meng et al. (2007)
	miR-106b	p21	Ivanovska et al. (2008)
	miR-130b	TP53INP1	Ma et al. (2010)
	miR-141	DLC-1	Banaudha et al. (2011)
	miR-155	SOX6	Xie et al. (2012)
	miR-191	SOX4, IL1A, TMC7	Elyakim et al. (2010)
	miR-216	TSLC1	Chen et al. (2012)
	miR-221	DDIT4 PTEN p27 p57	Pineau et al. (2010) Garofalo et al. (2009) Le Sage et al. (2007); Fornari et al. (2008); Pineau et al. (2010) Fornari et al. (2008)
	miR-222	PTEN p27	Garofalo et al. (2009) Le Sage et al. (2007); Pineau et al. (2010)
	miR-373	PPP6C	Wu et al. (2011)
	miR-423	p21Cip1/Waf1	Lin et al. (2011)
	miR-517a	Not determined	Toffanin et al. (2011)
Cell motility/Invasion (+)			
	miR-17-5p	Not determined	Yang et al. (2010)
	miR-21	RHOB PTEN	Connolly et al. (2010) Meng et al. (2007)
	miR-30d	GNAI2	Yao et al. (2010)
	miR-143	FNDC3B	Zhang et al. (2009)
	miR-151	RhoGDIA	Ding et al. (2010)
	miR-181b	TIMP3	Wang et al. (2010)
	miR-210	VMP1	Ying et al. (2011)
	miR-216	TSLC1	Chen et al. (2012)
	miR-221	PTEN, TIMP3	Garofalo et al. (2009)
	miR-222	PTEN, TIMP3 PPP2R2A	Garofalo et al. (2009) Wong et al. (2010)
	miR-517a	Not determined	Toffanin et al. (2011)
Cell viability (+)/apoptosis (-)			
	miR-15b	Bcl-w	Chung et al. (2010)
	miR-21	PTEN	Meng et al. (2007)
	miR-25	Bim	Li et al. (2009)
	miR-183	PDCD4	Li et al. (2010)
	miR-221	Bmf	Gramantieri et al. (2009)
	miR-221	PTEN	Garofalo et al. (2009)
	miR-224	API-5	Wang et al. (2008)
	miR-602	RASSF1A	Yang et al. (2010)

correlation between miR-122 and cyclin G1 expression and reported that miR-122 exerts its tumor suppressor ability by inhibiting HCC cell growth through the targeting of cyclin G1 (Gramantieri et al., 2007). Notably, miR-122 controls other factors involved in cell cycle progression, such as serum response factor (SRF), insulin-like growth factor 1 receptor (IGF1R), tyrosine-protein phosphatase non-receptor type 1 (PTPN1), or the members of the septin family SEPT2 and SEPT9 (Bai et al., 2009; Zeng et al., 2010a,b; Boutz et al., 2011). Furthermore, miR-122 may be involved in the regulation of the balance between proliferation and differentiation in hepatocytes by indirectly activating the expression of hepatic functional genes, such as cholesterol-7-alpha hydroxylase gene (CYP7A1) through the repression of the transcription repressor CUTL1, which is known to promote proliferation and suppress differentiation (Xu et al., 2010a,b).

MiRNAs can modulate cell proliferation by targeting a variety of other key factors involved in the control of cell cycle progression. For instance, miR-1 is frequently silenced in HCC through CpG-island methylation and capable of directly targeting and inhibiting of fork head box transcription factor (FoxP1), hepatocyte growth factor receptor (MET, c-MET, or HGFR), and histone deacetylase 4 (HDAC4) (Datta et al., 2008). Another example is miR-223, which down-regulates Stathmin 1 (STMN1), a key microtubule regulatory protein (Wong et al., 2008). Finally, let-7g may act as a tumor suppressor gene that inhibits HCC cell proliferation by down-regulating oncogene c-Myc and up-regulating the tumor suppressor gene p16 (INK4A) (Lan et al., 2011).

### Metastasis

Recent studies suggest an important role for miRNAs in metastatic formation. Accordingly, anti-metastatic-related miRNAs are frequently silenced in HCC. The MET signaling pathway plays an important role in the promotion of HCC invasion mechanisms. As reported above, the tumor suppressor miR-1, which is down-regulated in HCC, is a negative regulator of MET (Datta et al., 2008). By direct targeting of MET and inhibition of the MET-induced phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), miR-34a was also reported to suppress tumor invasion and migration in HCC patients (Li et al., 2009a,b). MiR-199a-3p, another miRNA with MET as a direct target, also inhibits the mammalian target of rapamycin (mTOR), leading to G1-phase arrest and invasiveness decline (Fornari et al., 2010). In addition, miR-23b is capable of decreasing the migration and proliferation abilities of HCC cells by targeting MET and the urokinase-type plasminogen activator (uPA) (Salvi et al., 2009). MiR-122 also exerts an anti-metastatic role in the liver, as reported by Boutz and colleagues, who demonstrated the correlation between miR-122, the matrix metalloproteinase (MMP) 7, and paxillin (PXN) (Boutz et al., 2011). Furthermore, miR-122 has been

described as a negative regulator of ADAM10 and ADAM17 (a disintegrin and metalloprotease family 10 and 17), both obviously involved in metastasis (Bai et al., 2009; Tsai et al., 2009).

LIN28B, a key RNA-binding protein highly expressed in HCC which regulates tumor formation and invasion through the coordinated repression of the let-7/miR-98 family and the induction of multiple oncogenic pathways in HCC, has also been reported to be inhibited by miR-125b (Liang et al., 2010). Recently, miR-139 was demonstrated to interact with the pro-invasion factor Rho-kinase 2 (ROCK2) (Wong et al., 2011). Accordingly, miR-139 is frequently down-regulated in HCC and associated with a poor prognosis and high metastatic features. The abnormal expression of the oncogene Six1 is generally associated with aggressive forms of cancers. Six1 has been shown to be repressed by miR-185, impeding anchorage-independent growth and cell migration in several kinds of tumors, including HCC (Imam et al., 2010). Another tumor suppressor miRNA that represses the metastatic characteristics of hepatocarcinoma cells is miR-193b. MiR-193b was reported to inhibit the invasion and migration of HCC cells by targeting cyclin D1 and the oncogene ETS1 (Xu et al., 2010a,b). More recently, miR-142-3p has been included in the anti-metastatic miRNA group because of its ability to target RAC1, a factor that regulates diverse cellular events, including migration and invasion (Wu et al., 2011a-c).

### Cell viability/apoptosis

Typically, anti-apoptotic factors are known to be frequently the target of tumor suppressor miRNAs. For instance, let-7c or let-7g negatively regulates the expression of the anti-apoptotic Bcl-xL by targeting its 3'-UTR in both Huh-7 and HepG2 cell lines (Shimizu et al., 2010). This suggests that the low expression of let-7 may contribute to the augmentation of Bcl-xL commonly observed in liver cancer. Interestingly, the over-expression of let-7-c significantly enhances the apoptosis of HCC cells induced by Sorafenib. In a similar manner, it has been demonstrated that the forced expression of miR-101, normally down-regulated in HCC cell lines and HCC tumors, can exert a pro-apoptotic action by targeting Mcl-1 (Su et al., 2009).

The hepatospecific miR-122 has also been identified to be capable of directly targeting other anti-apoptotic Bcl-2 family members and subsequently to reduce HCC cell viability (Lin et al., 2008). In a therapeutic perspective, Fornari and co-workers evaluated the effect of restoring miR-122 expression on triggering chemotherapy-induced apoptosis using doxorubicin and demonstrated that miR-122 increased sensitivity to the treatment (Fornari et al., 2009). Moreover, the patients who underwent a surgical resection and displayed lower levels of miR-122 were associated with a shorter time to recurrence. Xiong and co-workers also highlighted a reduced expression of miR-29 in HCC tissues that was

related with poor survival rates (Xiong et al., 2010). In this study, the enhancement of miR-29 expression dramatically increased HCC cell sensitivity to various apoptotic signals through the direct targeting of both the anti-apoptotic Bcl-2 and Mcl-1.

## Oncomirs

### Proliferation

Oncogenic miRNAs are generally ubiquitously expressed in normal tissues but are highly enriched in tumors. Obviously, miR-221 and miR-21 appeared as relevant oncomirs responsible for the promotion of HCC growth. First, these miRNAs present high expression levels in liver tumors and HCC cell lines and exhibit the property of inhibiting the tumor suppressor phosphatase and tensin homolog (PTEN), contributing to HCC growth and spread (Meng et al., 2007; Garofalo et al., 2009). Experimental inhibition of miR-21 increases the expression of PTEN and abolishes HCC cell line proliferation and invasiveness. Furthermore, miR-221 can also modulate the cell cycle by directly targeting two cyclin-dependent kinase inhibitors (CDKI) CDKN1B/p27 and CDKN1C/p57 (Fornari et al., 2008). Thus, the up-regulation of miR-221 observed in cancer cells leads to the promotion of HCC growth by increasing the entry in the S-phase through the control of these two CDKIs. Next, miR-221 was identified as a negative regulator of the DNA damage-inducible transcript 4 (DDIT4), a modulator of the mTOR pathway (Pineau et al., 2010). More recently, miR-373 was found to be up-regulated in human HCC tissues but not in adjacent normal tissues. This miRNA exerts its oncogenic activity by promoting cell proliferation through the inhibition of the protein phosphatase 6 catalytic subunit (PPP6C), a negative cell cycle regulator that regulates the G1-S phase transition (Wu et al., 2011a-c).

### Metastasis

The most representative miRNA globally exhibiting a persistent over-expression in all solid tumors, where it acts as an oncogene, is miR-21. As reported above, miR-21 mainly exerts its oncogenic activity in HCC by modulating PTEN expression and the PTEN-dependent pathways, mediating the phenotypic characteristics of cancer cells, such as proliferation, migration, and invasion (Meng et al., 2007). The augmentation of miR-21 expression is often associated with the poor differentiation of the tumor. The ability to target PTEN and to inhibit its expression is not exclusive to miR-21, as miR-221 and miR-222 can also significantly repress the expression of this major tumor suppressor. In addition, miR-221 and miR-222 can regulate the expression of the protein phosphatase 2A subunit B (PPP2R2A) and TIMP3, an inhibitor of metalloproteases. Thus, miR-221 and miR-222 over-expression enhances cellular migration through the activation of the

AKT pathway and metalloprotease expression (Garofalo et al., 2009; Wong et al., 2010). The induction of the enzymes responsible for the degradation of the extracellular matrix represents a key event for promoting the invasive process. Wang and colleagues demonstrated the induction of miR-181b by transforming growth factor (TGF)-beta and the enhancement of the activity of MMP2 and MMP9 through the decrease of TIMP3, thus promoting HCC cell invasiveness (Wang et al., 2010).

Other pathways or cellular processes responsible for cancer cell invasiveness can be modulated by the pro-metastatic miRNAs. First, the member of the oncogenic miR-106b family, miR-17-5p, has been reported to be over-expressed in liver cancer, leading to the enhancement of HCC cell migration and proliferation through a mechanism that involves the activation of the p38 mitogen-activated protein kinase MAPK pathway and an increased phosphorylation of heat shock protein 27 (HSP27) (Yang et al., 2010a,b). A study performed using a metastatic HBV-related HCC cell model demonstrated that nuclear factor NF-kappaB mediated the increased expression of miR-143, a miRNA that favors the invasive and metastatic behavior of liver tumor cells by repressing FNDC3B (Zhang et al., 2009). The pro-metastatic miR-151, frequently amplified on 8q24.3 and co-expressed with the host gene FAK, significantly increases tumor invasion and metastasis by directly targeting RhoGDI A, a putative metastasis suppressor in HCC, leading to the activation of Rac1, Cdc42, and Rho GTPases. MiR-151 also functions synergistically with FAK to enhance HCC cell motility and spreading (Ding et al., 2010). Recently, miR-517a has been identified as an over-expressed miRNA in liver cancer that promotes tumorigenesis and metastatic dissemination (Toffanin et al., 2011).

### Cell viability/apoptosis

As previously exposed, tumor suppressor miRNAs exert a pro-apoptotic activity by targeting anti-apoptotic factors such as Bcl-xL or Mcl-1. Conversely, oncomirs participate in tumorigenesis and carry anti-apoptotic effects through the negative modulation of the pro-apoptotic members of the Bcl-2 family. Gramantieri and collaborators revealed that HCC tissues exhibit an inverse correlation between miR-221 and the expression of Bmf, as well as a direct correlation between Bmf and the activated caspase-3 (Gramantieri et al., 2009). *In vitro*, the enforced expression of miR-221 causes the down-regulation of Bmf, whereas miR-221 silencing induces Bmf up-regulation and leads to an increase of apoptotic cell death. MiR-25, a member of the miR-106b-25 cluster that is over-expressed in HCC, was also evidenced to exert an anti-apoptotic effect by targeting and inhibiting the pro-apoptotic factor Bim (Li et al., 2009a,b). On the other hand, miRNAs can also regulate programmed cell death by targeting other apoptosis-related genes. For example, miR-183 was identified to be frequently up-regulated in HCC tissue samples and targeting programmed cell death 4 (PDCD4), a pro-