MicroRNA involvement in hepatocellular carcinoma

Laura Gramantieri a, Francesca Fornari a, Elisa Callegari b, Silvia Sabbioni b, Giovanni Lanza b, Carlo M. Croce b, c, Luigi Bolondi a, Massimo Negrini b *

a Department of Internal Medicine and Gastroenterology, University of Bologna, Bologna, Italy
b Department of Experimental and Diagnostic Medicine, University of Ferrara, Ferrara, Italy
c Comprehensive Cancer Center, Ohio State University, Columbus, OH, USA

Received: August 30, 2008; Accepted: October 1, 2008

Abstract

Hepatocellular carcinoma (HCC) is the third cause of cancer-related death worldwide. Curative options for HCC are limited and exclusively available for patients carrying an early stage HCC. In advanced stages, traditional chemotherapy proved to be only marginally effective or even toxic. Thus, the identification of new treatment options is needed. New targets for non-conventional treatment will necessarily take advantage of progresses on the molecular pathogenesis of HCC. MicroRNAs (miRNAs) are a group of tiny RNAs with a fundamental role in the regulation of gene expression. Aberrant expression of several miRNAs was found to be involved in human hepatocarcinogenesis. miRNA expression signatures were correlated with bio-pathological and clinical features of HCC. In some cases, aberrantly expressed miRNAs could be linked to cancer-associated pathways, indicating a direct role in liver tumourigenesis. For example, up-regulation of mir-221 and mir-21 could promote cell cycle progression, reduce cell death and favour angiogenesis and invasion. These findings suggest that miRNAs could become novel molecular targets for HCC treatment. The demonstration of in vivo efficacy and safety of anti-miRNA compounds has opened the way to their use in clinical trials.

Keywords: microRNA • hepatocellular carcinoma • diagnosis • therapy

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, where it represents the third cause of cancer-related death [1]. Because of differences in risk factors, regional incidence is widely variable. It ranges from 5.5 to 14.9 per 100,000 individuals, but it may reach 100 per 100,000 in some regions of Africa and South East Asia. Hepatitis B virus (HBV) infection is the main risk factor in Asia and Africa, whereas Hepatitis C virus (HCV) infection is the main risk factor in western countries and in Japan. Other conditions increasing the risk of chronic liver disease and HCC development are alcohol abuse, aflatoxin B1 or vinyl chloride exposure, primary biliary cirrhosis, diabetes, non-alcoholic fatty liver disease and genetic disorders like haemochromatosis and α1-antitrypsin deficiency. Many of these factors are known causes of liver cirrhosis, which represents a pre-neoplastic condition for HCC. Indeed, in 80–90% of cases, HCC arises on a background of cirrhosis. The remaining HCCs, which arise in an otherwise healthy liver, are thought to develop, at least in part, from the malignant transformation of liver adenomas [2].
A worldwide accepted staging system for HCC on cirrhosis, able to provide the proper guide for treatment and overall management of cirrhotic and HCC patients, is lacking. Among the several proposed HCC classification systems, the Barcelona Clinic Liver Cancer (BCLC) (Table 1) is one of the most used, as it offers the advantage of linking tumour staging to the best therapeutic strategy [3].

Treatment strategy is tailored on the basis of the extent of tumour burden, liver function, physical status and potential treatment efficacy. In very early and early stage HCC, potential curative treatments are available. They include surgical resection, percutaneous ablation and liver transplantation. However, only about 30–40% of cirrhotic patients enrolled in surveillance programs are eligible for these types of intervention [4] and, even after a curative treatment, the recurrence rate approaches 70% at 5 years. In advanced HCC, treatment options are even more limited: curative treatments are not available and traditional chemotherapy proved to be only marginally effective or even toxic in either adjuvant or neo-adjuvant settings.

Thus, the identification of new possible targets for the development of non-conventional treatments is urgent and will necessarily take advantage of progresses in the comprehension of the molecular pathogenesis of HCC [5].

### Molecular pathways and biological functions altered in hepatocarcinogenesis

HCC results from the deregulation of multiple intracellular and extracellular signalling pathways. Initial steps involve the disruption of a set of interdependent pathways controlling the homeostasis between cell growth and apoptosis. At later stages, cells may acquire angiogenic, invasive and metastatic properties, in a process that involves the interactions of neoplastic cells with the surrounding microenvironment. To develop the above cancer traits, several elements of the Retinoblastoma (RB), p53, rat sarcoma virus oncogene (RAS), wingless-type (WNT) and transforming growth factor (TGF)-β pathways have been frequently found to be genetically or epigenetically altered in HCC.

The cell membrane receptor tyrosine kinase and the downstream RAS-mitogen-activated protein kinase and phosphatidylinositol 3-kinase (PI3K)-AKT kinase signalling pathways are activated by several growth factors whose role in chronic liver diseases and HCC development is established [6]. By binding to their cell surface receptors, fibroblast growth factor (FGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), TGF-α, epidermal growth factor (EGF) and vascular-endothelial growth factor (VEGF) activate these pathways and ultimately drive cell proliferation and promote cell survival, but also trigger angiogenesis, invasion and metastasis. Overexpression of met proto-oncogene (MET), the HGF receptor, was found in 40–70% of HCCs [7–10]. Overexpression of VEGF was also detected [11, 12] and linked to advanced cancer phenotype [13–17]. Together with VEGF, overexpression of FGF was associated with angiogenic and invasive phenotypes [18–20]. Overexpression of PDGF and its receptor have also been associated with HCC pathogenesis [21, 22]. Among downstream effectors, overexpression of RAS has been demonstrated in HCC [23], while the presence of activating point mutations was infrequently detected [24, 25].

The Wnt/β-catenin is another signalling pathway that is frequently activated in hepatocarcinogenesis [26]. Its activation leads to β-catenin stabilization, enabling its translocation to the nucleus, where it interacts with T-cell-specific transcription factor (TCF/LEF) transcription factors, which, in turn, activate the transcription of target genes including c-myc, c-met, cyclin D1, VEGF, metalloproteases and others. The Wnt-β-catenin pathway was found to be abnormally activated through several mechanisms in HCC: gain of function mutations at the β-catenin N-terminus in 12–26% of HCCs [27], deletions or mutations or epigenetic alterations of E-cadherin gene, loss of function mutations at the AXIN1 or AXIN2 genes in 8–13% of HCCs [27, 28]. In addition to these mutation events, the increased stability of β-catenin may be related to phosphorylation and consequent inactivation of GSK-3β by growth factors, among which insulin, insulin-like growth factor (IGF)-1, IGF-2, EGF, PDGF, HGF, TGF-β and tumour necrosis factor (TNF)-α. A stabilization of β-catenin may also result from Erk-primed inactivation of GSK-3β or the action of HBV-X protein [29]. Recently, mutations of the β-catenin gene have been described in liver adenomas with a higher risk of malignant transformation [30].

| Stage       | Features                                                                                   |
|-------------|-------------------------------------------------------------------------------------------|
| Very early  | Well-differentiated tumours less than 2 cm in size, in cirrhotic patients with well-preserved liver function |
| Early       | Tumours within the Milan criteria (a single HCC less than 5 cm or three HCC nodules less than 3 cm each, [158]) in patients with preserved liver function. |
| Intermediate| Large, multifocal tumours in patients with preserved liver function, without cancer-related symptoms and macrovascular invasion or extrahepatic spread |
| Advanced    | Large, multifocal tumours in patients with mild cancer-related liver dysfunction and/or vascular invasion or extrahepatic spread |
| End stage   | Tumours with extensive liver involvement, depressed physical status and/or liver function |

Table 1 Hepatocellular carcinoma classification according to the BCLC staging system
Cell proliferation and survival are main outcomes of signal transduction pathways, but several other downstream effectors are also aberrant in cancer. For example, Rb1, cyclin-dependent kinases (CDKs), cyclins and CDK inhibitors, which directly act coordinately to regulate cell cycle, have been extensively examined in HCC. In HCC, inactivation of the RB1 gene may occur through either loss of chromosome 13, found in about 30% of HCCs [31, 32], or epigenetic mechanisms [33], while point mutations are rare. However, RB1 protein inactivation may derive from the aberrant expression of Gankirin, a protein able to bind RB1 and increase RB1 phosphorylation and its degradation by the proteasome. Gankirin was found up-regulated in all HCCs [34].

Furthermore, among cell cycle controlling elements, cyclins were reported to be up-regulated, while cell cycle negative regulators were often decreased in HCC tissue, compared with surrounding parenchyma [26]. CyclinD1/CDK4 is overexpressed in about 60% of HCCs [35]. Among CDK inhibitors, p16/INK4A, which specifically targets CDK4 and CDK6 thus inactivating CDK4/cyclin D1 complexes [36], is functionally inactivated in a large fraction of HCCs due to deletions at the short arm of chromosome 9 in about 20% of HCCs [27, 31, 32] and methylation of p16/INK4A promoter in 30–70% of cases [37, 38]. Two other proteins acting as tumour suppressor genes in HCC and directly regulating the cell cycle progression are the cyclin-dependent kinase inhibitors of the CDK suppressor genes in HCC and directly regulating the cell cycle progression have been extensively described in HCC, and has been shown to vary according to different geographic areas, possibly reflecting different etiologic mechanisms. The G to T transversion of codon 249 of TP53 gene is the typical alteration found in about 50% of HCCs consequent to aflatoxin B1 exposure [58]. Conversely, mutations of TP53 unrelated to aflatoxin B1 exhibit a lower frequency (10–30%) [27] and are more frequent in advanced cases. In addition to TP53 mutations, several other factors involved in hepatocarcinogenesis have been reported to impair p53 function. In particular, interactions between p53 and HBV-X protein lead to a reduced p53-sequence-specific DNA binding activity, thus reducing p53 transcriptional activity and blocking p53-mediated apoptosis. Concerning HCV, a modulation of p53 promoter transcriptional activity was demonstrated in HCC-derived cell lines and was ascribed to HCV-core protein [59].

Advanced tumor features include the ability of cancer cells to promote uncontrolled angiogenesis and invade tissues and blood vessels. In this regard, HCC is one of the most vascular solid tumours with high propensity for vascular invasion. The above-mentioned aberrant signalling pathways are functional to this feature. Angiogenesis plays a crucial role in HCC development, growth and metastasis and is used as a diagnostic criterion. The switch to an angiogenic phenotype is triggered by the activation of genes like RAS, inactivation of p53, or cellular stress factors like hypoxia, reactive oxygen species and nutrient deprivation.

Signalling pathways crucial for the angiogenic process include growth-factor-mediated pathways such as VEGF and FGF receptor signalling as well as the nitric oxide signalling. VEGF expression is stimulated by hypoxia through the hypoxia inducible factor-1 (HIF-1) and the IGF-2 and it is produced by both endothelial and tumour cells. VEGF exerts a mitogenic effect on endothelial cells and increases vascular permeability. The role of VEGF in the development of neovascularization of HCC has been extensively reported in the transition between low-grade dysplastic nodules to high-grade dysplastic nodules to early HCC. Furthermore VEGF expression correlates with HCC progression, metastatization, tendency towards portal invasion and higher recurrence rate.

In addition to angiogenesis, another distinguished feature of HCC is its propensity towards vascular and tissue invasion. One of the signalling pathways conferring invasive potential to HCC cells, is mediated by the HGF/MET axis. HGF is the most potent growth factor for hepatocytes and, by binding to its transmembrane
tyrosine kinase receptor, MET, it promotes proliferation and regeneration, migration, survival and angiogenesis and it is involved in the control of invasive growth both during tumourigenesis and in embryonic development [60, 61]. Cytoplasmic downstream effectors of HGF/MET signalling axis include phospholipase Cγ (PLCγ), STATs, PI3K, ERK-1/2 [62]. Among genes targeted by HGF/MET signalling, MMPs and urokinase-type plasminogen activator are thought to play an important role in cell migration [63].

As exemplified above, significant and complex cross-talks among the different pathways exist and are involved in different aspects of HCC development and progression. These cross-talks, largely not understood at the molecular level, could potentially account for the resistance to molecularly targeted drugs, which are able to hit pathways only at one or few sites. In this context, the peculiar function of miRNAs may be important. Indeed, since the expression of many different proteins is affected by the
deregulation of a single miRNA, the targeting of even a single deregulated miRNA for therapeutic purposes may have an effect on several cancer-associated pathways. Thus, recognizing the deregulated miRNAs and their protein-coding gene targets is therefore a relevant task for understanding the molecular basis of liver tumourigenesis and for the development of potentially useful diagnostic and therapeutic tools.

Numerous miRNAs are aberrantly expressed in human HCC

In the recent years, several studies revealed that the expression of miRNAs is deregulated in human HCC in comparison with matched non-neoplastic tissue [64–73]. The list of aberrantly expressed miRNAs in HCC identified in various studies is reported in Table 2. Among these, some miRNAs were identified as aberrantly expressed by more than one study (Table 3), thus indicating that irrespective of the employed technological platform or set of samples, these miRNAs were the most likely involved in liver tumourigenesis. In addition and in support of this hypothesis, among aberrantly expressed miRNAs in HCC, some were also deregulated in other neoplasms. For example, the up-regulation of the miR-221/222 was also reported in colon, pancreas, stomach, bladder carcinomas and glioblastomas, or miR-21 was also reported in ovarian, lung, breast carcinomas and glioblastomas, while the down-regulation of miR-199a, miR-200b and miR-214 was reported in ovarian cancer and miR-199b in ovarian and lung cancer. In support of their role in liver tumourigenesis, gene targets, like the cyclin-dependent inhibitors p27/Cdkn1B and p57/Cdkn1C, or the P53K antagonist phosphatase and tensin homolog (PTEN), involved in cell cycle and cell death regulation were experimentally validated targets of miR-221/222 and miR-21.

Among the up-regulated miRNAs, miR-373 was also identified. This oncogenic miRNA was previously found to be up-regulated in testicular cancer and in breast cancer metastasis and protect cells from activated oncogenes [74, 75]. Interestingly, some miRNAs that are up-regulated in HCC, such as miR-21, miR-20, miR-213 and miR-181b, were also found to be up-regulated in hypoxic conditions. Thus, aberrant miRNA expression in HCC may lead to protection from various stress stimuli.

Among the down-regulated miRNA in HCC, several members of the large let-7 family and the miR-143/145 were found. Although for members of the let-7 family there are some controversial reports, these miRNAs emerged as consistently down-regulated in several human cancers [76–82].

Among the down-regulated miRNAs in HCC is the hepato-specific miR-122, which accounts for about 70% of the miRNA population in the adult liver, where it acts as a key regulator of cholesterol and fatty-acid metabolism. Significantly, the down-regulation of miR-122 was detected in more than 70% of HCC [73]. It was shown that the level of miR-122 expression increases in the mouse liver throughout development, to reach the maximum just before birth. Thus, the loss of expression of miR-122 of HCC cells may represent either a differentiation reversion or a block to a less differentiated status of liver cells. There are also indications that the down-regulation of miR-122 may have a more direct role in tumourigenesis through the activation of cyclin D1 [73].

Biological and molecular functions affected by miRNA deregulated in HCC

The role of miRNAs either as oncogenes or tumour suppressors in human cancer has been established [83, 84]. Various studies have also begun to elucidate the molecular functional links between miRNA abnormal expression and the hallmarks of malignant transformation: aberrant cell growth, cell death, differentiation, angiogenesis, invasion and metastasis [85]. Here, we summarize the available evidence in HCC.

As previously indicated, MET, the tyrosine kinase HGF receptor, is overexpressed in 40 to 70% of HCCs and is involved in cell motility, invasion and metastasis. Recent reports indicated that MET is post-transcriptionally regulated by miR-199a/a* and miR-1 [86, 87] (Fig. 1). Genes for both miRNAs are methylated in HCCs. Members of the miR-199 family emerged in several studies as frequently down-regulated in HCC (see Table 3). Its in vivo significance needs to be confirmed, as the studies were confined to cell lines and the miRNA that was active on MET was the presumed ‘non-mature’ miR-199a* strand. In spite of the fact that the aberrant down-regulation of miR-1 did not emerge in several miRNA expression studies in HCC, it was recently shown that its expression was silenced in HCC cell lines and primary tumours via promoter methylation; in addition, miR-1 ectopic expression could induce apoptosis and inhibit cell cycle in HCC cell lines. Thus, the miR-1 silencing in HCC may remove a restriction element that permits MET overexpression.

The activation of tyrosine kinase receptors (RTKs) initiates a downstream cascade of events that lead cells to proliferate. Crucial elements of this signalling transduction pathway are members of the RAS family of oncogenes. A molecular link between miRNA deregulation and RAS expression has been established. The 3’ untranslated regions (UTRs) of the KRAS, NRAS and HRAS mRNAs contain multiple complementary sites for binding of let-7 members, and forced expression of let-7 in human cancer cells reduces RAS protein levels [78] (Fig. 1). Since let-7 is generally down-regulated in several human cancers, this mechanism could lead to the activation of the RAS pathway. As previously mentioned, it is interesting to note that a frequent overexpression but not point mutations of RAS has been reported in HCC.

Let-7 has also been shown to repress the high-mobility group AT-hook 2 (HMGA2) oncogene [79, 80], which encodes for a high-mobility group protein oncogenic in a variety of tumours, including benign mesenchymal tumours and lung cancers. The effect of let-7 on HMGA2 was dependent on multiple target sites
| miRNA   | Expression in HCC | miRNA cluster                      | Chrom  |
|---------|-------------------|------------------------------------|--------|
| let-7a-1| Down              | let-7-a1/let-7f1/let-7d            | 9q22   |
| let-7a-2| Down              | miR125b-1/let7a-2/miR100           | 11q24  |
| let-7a-3| Down              | let-7-a3/let-7b                    | 22q13  |
| let-7b  | Down              | let-7-a3/let-7b                    | 22q13  |
| let-7c  | Down              | mir-99a/let-7c/125b-2              | 21q11  |
| let-7d  | Down              | let-7-a1/let-7f1/let-7d            | 9q22   |
| let-7e  | Down              | mir-99b/let7-e/125a                | 19q13  |
| let-7f-2| Down              | let-7f-2/mir-98                    | Xp11   |
| let-7g  | Down              |                                    | 3p21   |
| miR-101 | Down              |                                    | 1p13   |
| miR-122 | Down              |                                    | 18q21  |
| miR-125a| Down              | mir-99b/let7-e/125a                | 19q13  |
| miR-125b-1| Down           | miR125b-1/let7a-2/miR100           | 11q24  |
| miR-130 | Up                |                                    | 11q12  |
| miR-130a| Down              |                                    | 11q12  |
| miR-132 | Down              | mir-212/132                        | 17p13  |
| miR-135a| Up                |                                    | 12     |
| miR-136 | Down              | mir-770/493/337/431/432/127/432/136/370 | 14q32  |
| miR-139 | Down              |                                    | 11     |
| miR-143 | Down              | mir-143/145                        | 5q32–33|
| miR-145 | Down              | mir-143/145                        | 5q32–33|
| miR-150 | Down              |                                    | 19q13  |
| miR-18  | Up                | mir-17–92                          | 13q31  |
| miR-181b-1| Up               | mir-181-a1/181-b1                  | 1q31–32|
| miR-195 | Down              | mir-497/195                        | 17p13  |
| miR-199a-1-5p| Down           |                                    | 19p13  |
| miR-199a-2-5p| Down           | mir-199a2/214                      | 1q24   |
| miR-199b| Down              |                                    | 9q34   |
| miR-200a| Down              | mir-200b/200a/429                  | 1      |
| miR-200b| Down              | mir-200b/200a/429                  | 1p36   |
| miR-21  | Up                |                                    | 17q23  |
| miR-210 | Up                |                                    | 11p15  |
| miR-213 | Up                | mir-181-a1/181-b1                  | 1q31–32|
| miR-214 | Down              | mir-199a2/214                      | 1q23   |
| miR-221 | Up                | mir221/222                         | Xp11   |
| miR-222 | Up                | mir221/222                         | Xp11   |
| miR-223 | Down              |                                    | Xq12–13|
| miR-224 | Up                | mir224/452                         | Xq     |
| miR-301 | Up                | mir-301/454                        | 17     |
| miR-33  | Up                |                                    | 22q    |
| miR-34  | Up                | mir-34b/34c                        | 11q    |
| miR-373 | Up                | mir-371/372/373                    | 19q    |
| miR-376a| Up                | Cluster 34 miRNA                   | 14q32  |

Results are summarized from the following references: [65–73].
in the 3’UTR. It was reported that chromosomal translocations associated with human tumours disrupt repression of HMGA2 by let-7 miRNA, thus demonstrating that disruption of a single miRNA-target interaction and loss of miRNA-directed repression represent mechanisms for the activation of endogenous proto-oncogenes [79]. The disrupted repression of HMGA2 promotes anchorage-independent growth and the growth-suppressive effect of let-7 on lung cancer cells was rescued by overexpression of the HMGA2 ORF without a 3’UTR [79]. The link between the down-regulation of let-7 and the simultaneous up-regulation of HMGA2 was functionally associated with a cancer stem cell signature, at least in the breast cancer cells SKBR3, by Yu et al. [82]. These important findings need to be assessed in HCC too. The importance of let-7 down-regulation in cancer is further supported by studies by Takamizawa et al. and Akao et al. [76, 81], who showed that let-7 can suppress the growth of A549 lung cancer cells and DLD-1 colon cancer cells in vitro.

A direct role of miRNAs in controlling cell growth by acting on elements of the cell cycle machinery was provided by studies on miR-221. MiR-221, which was recently shown to be induced by MYCN [88] and repressed by p53 [89], emerged as a significantly up-regulated miRNA in glioblastoma, pancreatic, hepatocellular, kidney, bladder, prostate and thyroid cancer [44–46, 73, 79, 90–94], thus suggesting an oncogenic role in several human neoplasms. Its oncogenic function was substantiated by the discovery of its ability to modulate the expression of the cyclin-dependent kinase inhibitor CDKN1B/p27, a key controller of cell cycle progression [46, 93]. More recently, another cyclin-dependent kinase inhibitor, CDKN1C/p57, was also shown to be target of miR-221 [44, 45], strengthening the role of miR-221 in promoting cell cycle progression (Fig. 1). Moreover, the BH3-only protein BMF was recently proven to be a target of miR-221 (Gramantieri and Negrini, manuscript in preparation). Through this mechanism, miR-221 could protect cells from ‘anoikis’, a form of Apoptosis induced by the detachment of anchorage-dependent cells from the surrounding extracellular matrix. Evading anoikis is a critical step in the process of metastasis [95, 96]. Taken together, the finding that miR-221 could promote cell cycle progression and protect cells from apoptosis outlines the importance of the aberrant expression of one miRNA in cancer, whose action on various targets could simultaneously affect multiple tumourigenic pathways.

Signal pathways from activated RTKs include also the PI3K AKT. The activation of this pathway leads to the activation of AKT kinases, which phosphorylate several protein targets that in turn

| microRNA class | microRNA | Expression in HCC | miRNA cluster | Chrom | Other cancers | References |
|----------------|----------|------------------|---------------|-------|--------------|------------|
| miR-18         | miR-18   | Up               | mir-17–92     | 13q31 | No           | [70, 159]  |
| miR-21         | miR-21   | Up               | -             | 17q23.2 | Ovarian, glioblastoma, lung, breast | [66, 90, 97, 135–137, 159, 160] |
| miR-221        | miR-221  | Up               | mir221/222    | Xp11.2 | Colon, pancreas, stomach, bladder, glioblastoma, thyroid | [69, 73, 90, 91, 94, 97, 135, 159] |
| miR-222        | miR-222  | Up               | mir221/222    | Xp11.2 | Stomach, pancreas | [66, 69, 97, 135] |
| miR-224        | miR-224  | Up               | mir224/452    | Xq     | Prostate, Thyroid | [66, 68, 70, 161, 162] |
| miR-122        | miR-122  | Down             | -             | 18q21  | No           | [66, 73, 97] |
| miR-125        | miR-125a | Down             | mir-99b/let7-e/125a | 19q13.4 | Breast, Ovarian, Lung | [70, 97, 137, 160, 163] |
| miR-125b-1     | miR-125b-1 | Down          | mir125b-1/let7a-2/miR100 | 11q24.2 | Breast, Ovarian | |
| miR-130a       | miR-130a | Down             | -             | 11q12  | Breast, Lung  | [73, 135, 159] |
| miR-150        | miR-150  | Down             | -             | 19q13  | No           | [73, 159] |
| miR-199        | miR-199a-1–5p | Down         | -             | 19p13.2 | Ovarian | [70, 73, 97, 136, 159, 160, 163] |
| miR-199a-2–5p  | miR-199a2/214 | Down         | 1q24.3        | Ovarian | |
| miR-199b       | miR-199b | Down             | -             | 9q34   | Ovarian, Lung | [70, 73, 159, 163] |
| miR-200        | miR-200a | Down             | mir-200b/200a/429 | 1p36.3 | No           | [70, 73, 159, 163] |
| miR-200b       | miR-200b | Down             | mir-200b/200a/429 | 1p36.3 | Ovarian     | |
promote cell survival. This pathway is controlled by the tumour suppressor lipid-phosphatase PTEN. It was shown that PTEN is a direct target of miR-21 [97] (Fig. 1), a miRNA that is frequently overexpressed in most types of human cancers. Thus, PTEN could be repressed by overexpression of miR-21, which would lead to cell survival through PI3K-AKT pathway activation.

miR-21 can also down-regulate the tumour suppressor programmed cell death 4 (Pdcd4) [98, 99]. Pdcd4 is believed to have a role in TGF-β induced apoptosis. However, it may also have other functions. It is up-regulated in senescent fibroblasts and it may inhibit proliferation, possibly through the indirect suppression of CDK1/cdc2 kinase. Moreover, it acts as a negative regulator of invasation, initial step for cancer cell metastasis. Anti-miR-21-transfected RKO cells showed an increase of Pdcd4-protein and reduced invasion, while overexpression of miR-21 in Colo206f significantly reduced Pdcd4-protein amounts and increased invasion. Analyses of primary colorectal cancers revealed that an inverse correlation between miR-21 and Pdcd4-protein exists, suggesting that miR-21/Pdcd4 interaction may be relevant for invasion/invasation/metastasis of cancer cells.

miR-21 can also down-regulate the tropomyosin-1 [100], which suppresses anchorage-independent growth of MCF-7 breast cancer cells, further supporting the oncogenic miR-21 functions.

miR-373 is up-regulated in HCC. A link between miR-373 and p53 was proven by a study on miR-372 and miR-373, which were found to cooperate with oncogenic RAS to transform primary human cells [75]. The study proved that these miRNAs could confer protection to oncogene-activated p53 pathway. It was shown that primary human cells undergo growth arrest and senescence in response to mitogenic signals from oncogenes such as RAS, by the activation of the p53 pathway, a response that is reversed by the presence of non-functional p53. Voorhoeve et al. demonstrated that ectopic expression of miR372/373 was sufficient to allow transformation in the presence of wt p53. Thus the study demonstrated that miR372/373 confers protection to oncogene-activated p53 pathway. Interestingly, this is a characteristic found in testicular germ cell tumours, where, in contrast with other types of tumours, the miR-372/373 cluster is indeed highly expressed in a generally wt-p53 background, suggesting a role in the development of these tumours.

As previously mentioned, miR-122 is down-regulated in more than 70% of HCCs. A molecular investigation revealed that cyclin G1 is a direct target of miR-122. Cyclin G1 was initially identified as a transcriptional target of p53 [101–104]. Later, it was found that cyclin G1 can exert a negative regulation on p53 tumour suppressor gene by recruiting the B' subunit of PP2A phosphatase to dephosphorilate and activate Mdm-2, thus leading to p53 degradation [105, 106]. As a result, cyclin G1 overexpression can enhance cancer cell growth and its silencing suppresses cell proliferation [107–109]. In a mouse model, the absence of cyclin G1 was associated with a lower susceptibility to develop liver tumours, which was associated with an increased p53 tumour suppressor activity [110]. The up-regulation of cyclin G1 consequent to miR-122 down-regulation in human HCC may thus lead to p53 down-regulation and promote tumourigenesis.

**miRNA expression profiling in HCC classification and prognostic stratification**

miRNA expression could also be used to improve our ability to classify HCC and stratify their prognostic risk. The presently used classifications for HCC are exclusively based on clinical parameters [3, 111–116]. Tumour status (number and size of nodules, presence of vascular invasion and extrahepatic spread), liver function indicators, presence of portal hypertension and performance status are considered for prognostic purposes and for the choice of treatment. Because HCC is heterogeneous both from molecular and clinical perspectives [117, 118], the available classifications for HCC could be improved and redefined by the incorporation of molecular data.

Various molecular factors have been found to correlate with clinical parameters. For example, MET-regulated expression signature defines a subset of human HCCs with poor prognosis and aggressive phenotype [119]. CDKN1B/p27 and CDKN1C/p57 exhibits a relevant prognostic significance in human HCC. CDKN1B/p27 down-regulation is associated with advanced tumour stage, lower survival and higher recurrence rate of small HCC, higher biological aggressiveness, poor differentiation, portal invasion and high proliferative activity [120, 121]. Reduced CDKN1C/p57 labelling index was associated with worse outcomes and lower disease-free survival after surgery, suggesting that CDKN1C/p57 down-regulation might contribute to the progression of HCC through modulation of cell growth [39, 122]. Overexpression of the anti-apoptotic gene bcl-xl [53] independently predicts a decreased overall- and disease-free survival [54, 55]. High throughput technologies have made possible to explore gene expression patterns on large series and to characterise molecular signatures associated with different aetiologies [123–125], stage [126, 127], propensity to recurrence [128–130] and prognosis [131, 132]. Incorporation of these findings in HCC classification could be valuable in improving our ability to stratify HCCs; however, additional validations are still required before their clinical use is granted.

In this context, more recently, patterns of miRNA expression were found to correlate with bio-pathological and clinical parameters, indicating that miRNAs could become useful molecular markers for HCC classification and prognostic stratification. Among deregulated miRNAs, up-regulation of miR-221 was associated with shorter time to recurrence (Gramantieri and Negri, unpublished). This is not unexpected, giving the fact that miR-221 regulates the expression of p27 and p57 [44], two tumour suppressor proteins whose down-regulation was associated with poor prognostic factors in HCC [39, 120–122]. Recently, up-regulation of miR-210, one of the miRNA that is induced under hypoxia [133],
was associated with reduced disease-free and overall survival in breast cancer [134]. Since miR-210 is also up-regulated in HCC [97] as well as in a variety of solid cancers [135–138], its prognostic value may possibly be relevant for other cancers too, including HCC. The study by Ladeiro et al. [66] could identify miRNA signatures able to classify liver samples according to histology (tumour/non-tumour; benign/malignant; inflammatory adenomas and focal nodular hyperplasia), aetiology (alcohol consumption or HBV infection) and cancer gene mutations (β-catenin and hepatocyte nuclear factor 1). The study by Budhu et al. [72] revealed a 20-miRNA signature associated with venous invasion (Table 4). Significantly, the same signature could also correlate with disease-free and overall survival. The results by Budhu et al. may be particularly useful to classify patients with HCC at early stage, which may provide a more rational approach to treatment intervention. Hence, either alone or in combination with other parameters, miRNA expression patterns may potentially become useful markers for HCC classification and prognostic risk stratification.

### miRNA in HCC therapy?

To identify novel therapeutic approaches for the treatment of HCC, targeting genes associated with molecular pathways involved in human tumourigenesis has become the most rational approach. In HCC, targeting RAS by antisense oligonucleotides could inhibit hepatocarcinogenesis by restoring the apoptotic pathway. Inhibition of MET could control migration and invasion of HCC cells [139]. The use of imatinib mesylate, a platelet-derived growth factor receptor (PDGFR) and other tyrosine kinases inhibitor, has been suggested [140–142]; however, in spite of initial positive results [143], phase 2 clinical trials did not reveal any significant efficacy [144, 145].

New molecularly targeted agents with a selective action and few side effects on residual liver function are under investigation: taking advantage of the progresses in understanding the molecular pathogenesis of HCC, kinases inhibitors, anti-angiogenic

---

**Table 4** miRNA signatures associated with hepatocellular carcinoma (HCC) metastasis and survival

| Metastasis-associated miRNA* | Survival-associated miRNA* | Expression in HCC | miRNA cluster | Chrom |
|-----------------------------|---------------------------|-------------------|---------------|-------|
| miR-185                     |                           | Up                | -             | 22q11 |
| miR-207                     |                           | Up                | -             | 9p21  |
| miR-219–1                   | Yes                       | Up                | -             | 6p21  |
| miR-338                     | Yes                       | Up                | mir-338/mir-657| 17q25 |
| let-7g                      |                           | Down              | -             | 3p21  |
| miR-1–2                     | Yes                       | Down              | mir-133a-1/mir-1–2 | 18q11 |
| miR-122                     | Yes                       | Down              | -             | 18q21 |
| miR-124a-2                  | Yes                       | Down              | -             | 8q12  |
| miR-125b-2                  | Yes                       | Down              | mir-99a/let7c/mir-15b-2 | 21q21 |
| miR-126                     | Yes                       | Down              | -             | 9q34  |
| miR-148a                    | Yes                       | Down              | -             | 7q15  |
| miR-148b                    | Yes                       | Down              | -             | 12q13 |
| miR-15a                     | Yes                       | Down              | mir-15a/mir-16–1 | 13q14 |
| miR-194                     | Yes                       | Down              | mir-194-1/mir-215 | 1q41 |
| miR-19a                     | Yes                       | Down              | mir-17/18a/19a/20a/19b-1/92–1 | 13q31 |
| miR-30a                     | Yes                       | Down              | mir-30a/30c-2  | 6q13  |
| miR-30c-1                   | Yes                       | Down              | mir-30e/mir-30c-1 | 1p34 |
| miR-30e                     | Yes                       | Down              | mir-30e/mir-30c-1 | 1p34 |
| miR-34a                     | Yes                       | Down              | -             | 1p36  |
| miR-9–2                     | Yes                       | Down              | -             | 5q14  |

* These data were from Budhu et al. [72]
compounds, mTOR inhibitors and other molecularly targeted agents have been and are currently under evaluation in phase 2 and 3 trials. At present, the only phase 3 clinical trial so far completed compared the oral multikinase inhibitor sorafenib, which is known to block the kinase activities of VEGFR, PDGFR and Raf [146], with placebo in patients with advanced HCC. Treated patients displayed a median overall survival of 10.7 months versus 7.9 months in the placebo group, setting the standards for future investigations [147, 148]. This moderate improvement in therapeutic efficacy and overall survival may represent a significant progress, as most of the studies did not rely upon molecular characterization of the different gene targets prior to inclusion in clinical studies, which might have aid the selection of the best target population for the different treatment options.

The discovery that miRNAs play an important role in hepatocarcinogenesis has laid the foundation for their exploitation for molecular therapy. Feasibility of the approach has been proved. Song et al. used anti-Fas siRNA to protect mice from induced acute liver injury and, similarly, Zender et al. used anti-caspase-8 siRNA to protect mice against Fas ligand-induced liver injury [149, 150]. Both of these studies demonstrated a survival benefit using siRNA approach without signiﬁcant side effects. These studies also confirmed a high hepatic uptake of siRNA following their systemic administration. Although several studies have established the potential usefulness of miRNA-based therapy in cancer [76, 81, 151, 152], up to now there has not been any report of using agents that mimic miRNAs in animal or clinical models. More recently, anti-miRNA oligonucleotides (AMOs) have been developed. Among miRNAs up-regulated in HCC, transfection of cultured glioblastoma and breast cancer cells with AMOs anti-miR-21 induced inhibition of miR-21 accompanied by suppression of cell growth, associated with increased apoptosis [153, 154]. MiR-21 is overexpressed in colangiocarcinoma and its inhibition by AMOs increases sensitivity to the chemotherapeutic agent gemcitabine [155]. Either cholesterol-bound oligonucleotides (antagomirs), or LNA-modified oligonucleotides (LNA anti-miR) were also found to be effective in stably suppressing miRNA activity in the in vivo condition [156, 157]. In addition, liver appeared to be the organ most efficiently and consistently targeted by intravenous injection of AMOs. Recently, a study performed in African green monkeys assessed safety and efficacy of the approach. Efficient silencing of miR-122 was achieved by three doses of 10 mg/kg LNA-anti-miR, leading to a long-lasting and reversible decrease in total plasma cholesterol without any evidence for associated toxicities or histopathological changes in the liver of the animals. Thus, by proving feasibility, safety and efficacy for the use of AMOs in a pre-clinical setting, these studies established the basis for their use as therapeutic molecules in clinical trials. The potentiality for therapeutic implementation of small RNAs or AMOs in clinical practice is enormous. Moreover, as far as treatment of liver and HCC is concerned, the limitations encountered for other organs related to an effective in vivo delivery of the drugs is partly overcome by the current clinical application of techniques able to deliver the drugs directly into the hepatic artery branches.

Concluding remarks

The discovery of aberrantly expressed miRNAs in HCC has helped to reveal novel mechanisms in liver tumourigenesis. Understanding molecular tumourigenesis has established the basis for the development of more rational classification and therapeutic approaches. In HCC, aberrantly expressed miRNAs could be associated with bio-pathological and clinical features, making miRNA expression a potentially useful tool for HCC classification and prognostic stratification, in particular in early HCC, where the availability of potentially curative approaches requires a more sophisticated diagnostic approach. Finally, by revealing which miRNA are up- or down-regulated in liver tumourigenesis, new potential therapeutic targets have been revealed. Since technological advancements have shown that the use of miRNAs or AMOs as potentially therapeutic molecules is feasible and safe, their experimentation may become an important area of clinical investigation in the years to come.

Acknowledgements

This study was supported by the Associazione Italiana per la Ricerca sul Cancro, Ministero dell’Università e della Ricerca, Ministero della Salute, Fondazione Cariplo Progetto NOBEL and Fondazione Carisbo.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005; 55: 74–108.
2. Rebourcet S, Bioulac-Sage P, Zucman-Rossi J. Molecular pathogenesis of focal nodular hyperplasia and hepatocellular adenoma. J Hepatol. 2008; 48: 163–70.
3. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005; 42: 1208–36.
4. Sangiovanni A, Del NInno E, Fasani P, De Fazio C, Ronchi G, Romeo R, Morabito A, De Francinis R, Coimolto M. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. Gastroenterology. 2004; 126: 1005–14.
5. Villanueva A, Toffanin S, Llovet JM. Linking molecular classification of hepatocellular carcinoma and personalized medicine: preliminary steps. Curr Opin Oncol. 2008; 20: 44–53.
6. Ito Y, Sasaki Y, Horimoto M, Wada S, Tanaka Y, Kasahara A, Ueki T, Hirano T, Yamamoto H, Fujimoto J, Okamoto E, Hayashi N, Hori M. Activation of mitogen-activated protein kinases/extracellular signal-regulated kinases in human hepatocellular carcinoma. Hepatology. 1998; 27: 951–8.
14. Boix L, Rosa JL, Ventura F, Castells A, Bruix J, Rodes J, Bartrons R. c-met mRNA overexpression in human hepatocellular carcinoma. *Hepatology.* 1994; 19: 88–91.

15. Suzuki K, Hayashi N, Yamada Y, Yoshihara H, Miyamoto Y, Ito Y, Ito T, Katayama K, Sasaki Y, Ito A, Kisida Y, Kashiwagi T, Fusamoto H, Kamada T. Expression of the c-met protooncone gene in human hepatocellular carcinoma. *Hepatology.* 1994; 20: 1231–6.

16. Grigioni WF, Fiorentino M, D’Errico A, Ponzetto A, Crepaldi T, Prat M, Comoglio FM. Overexpression of c-met protooncone gene and raised K67 index in hepatocellular carcinomas with respect to benign liver conditions. *Hepatology.* 1995; 21: 1543–6.

17. Ueki T, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E. Expression of hepatocyte growth factor and its receptor, the c-met protooncone, in hepatocellular carcinoma. *Hepatology.* 1997; 25: 619–23.

18. Sugano Y, Matsuoka K, Tahashi Y, Furukawa F, Mori S, Yamagata H, Yoshida K, Matsushita M, Nishizawa M, Fujisawa J, Inoue K. Distortion of autocrine transforming growth factor beta signal accelerates malignant potential by enhancing cell growth as well as PAI-1 and VEGF production in human hepatocellular carcinoma cells. *Oncogene.* 2003; 22: 2309–21.

19. Moon WS, Rhyu KH, Kang MJ, Lee DG, Yu HC, Yeum JH, Koh GY, Tarnawski AS. Expression of VEGF and angiopoietin 2: a key to high vascularity of hepatocellular carcinoma? *Mod Pathol.* 2003; 16: 552–7.

20. Hirohashi K, Yamamoto T, Uenishi T, Ogawa M, Sakabe K, Takemura S, Funakoshi A, Ando M, Takada S, Koike K. Activated N-ras gene Ras oncogene p21 expres- tion allelotype. *Cancer Res.* 1997; 57: 3643–6.

21. Challen C, Guo K, Collier JD, Cavanagh D, Bassendine MF. Frequent point mutations in codons 12 and 61 of ras oncogenes in human hepatocellular carcinomas. *J Hepatol.* 1992; 14: 239–44.

22. Schmitt M, Horbach A, Kubitz R, Frilling A, Haussinger D. Disruption of hepatocellular tight junctions by vascular endothelial growth factor (VEGF): a novel mechanism for tumor invasion. *J Hepatol.* 2004; 41: 274–83.

23. Chen Z, Han ZG. Insight into hepatocellular carcinogenesis at transcriptome level by comparing gene expression profiles of hepatocellular carcinoma with those of corresponding noncancerous liver. *Proc Natl Acad Sci USA.* 2001; 98: 15089–94.

24. Laurent-Puig P, Legoiq P, Bluteau O, Belghiti J, Franco D, Binot F, Monges G, Thomas G, Bioulac-Sage P, Zucman-Rossi J. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. *Gastroenterology.* 2001; 120: 1763–73.

25. Satoh S, Daigo Y, Furukawa Y, Kato T, Miwa N, Nishiwaki T, Kawaseo T, Ishiguro H, Fujita M, Tokino T, Sasaki Y, Imaoa S, Murata M, Shimano T, Yamaoka Y, Nakamura Y. AKIN1 muta- tions in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AKIN1. *Nat Genet.* 2000; 24: 245–50.

26. Ding Q, Xia W, Liu JC, Yang JY, Lee DF, Xia J, Bartholomeusz G, Li Y, Yan P, Li Z, Bargou RC, Qin J, Lai CC, Tsai FJ, Tsai CH, Hung MC. Erk associates with and primes GSK-3beta for its inactivation resulting in upregulation of beta-catenin. *Mol Cell.* 2005; 19: 159–70.

27. Zucman-Rossi J, Jeannot E, Nhyei JT, Scoazec JY, Guettier C, Rebouissou S, Bacq Y, Leteurtre E, Paradis V, Michalak S, Wendum D, Chiche L, Fabre M, Mellotte L, Laurent C, Partensky C, Castaing D, Zafren ES, Laurent-Puig P, Balabaud C, Bioulac-Sage P. Genotype-phenotype correlation in hepatocellular adenoma: new classification and relationship with HCC. *Hepatology.* 2006; 43: 515–24.

28. Boige V, Laurent-Puig P, Fouchet P, Flejou JF, Monges G, Bedossa P, Bioulac-Sage P, Capron F, Schmitz A, Olschwang S, Thomas G. Concerted nonsyntetic allelic losses in hyperploid hepatocellular carcinoma as determined by a high-resolution allelyotype. *Cancer Res.* 1997; 57: 1986–90.

29. Nagai H, Pinea P, Tiollais P, Buendia MA, Dejean A. Comprehensive allelotyping of human hepatocellular carcinomas. *Oncogene.* 1997; 14: 2927–33.

30. Lin Y, Shi CY, Li B, Soo BH, Mohammed-Ali S, Wee A, Oon CJ, Mack PD, Chan SH. Tumour suppressor p53 and Rb genes in human hepatocellular carcinoma. *Ann Acad Med.* 1996; 25: 22–30.

31. Higashitani H, Itoh K, Nagao T, Dawson S, Nonoguchi K, Kid T, Mayer RJ, Arli S, Fujita J. Reduced stability of retinoblastoma
protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas. Nat Med. 2000;6:96–9.

35. Joo M, Kang YK, Kim MR, Lee HK, Jang JJ. Cyclin D1 overexpression in hepatocellular carcinoma. Liver. 2001;21:89–95.

36. Hickman ES, Moroni MC, Helin K. The role of p53 and pRB in apoptosis and cancer. Curr Opin Genet Dev. 2002;12:60–6.

37. Weilbrauch M, Benicke M, Lehnert G, Wittke C, Wibitzky R, Tannapfel A. Frequent K-ras-2 mutations and p16(INK4a) methylation in hepatocellular carcinomas in workers exposed to vinyl chloride. Br J Cancer. 2001;84:982–9.

38. Matsuda Y, Ichida T, Matsuazawa J, Sugimura K, Asakura H. p16(INK4a) is inactivated by extensive CpG methylation in human hepatocellular carcinoma. Gastroenterology. 1999;116:394–400.

39. Ito Y, Takeda T, Sakon M, Tsujimoto M, Monden M, Matsuura N. Expression of p57/Kip2 protein in hepatocellular carcinoma. Oncology. 2001;61:221–5.

40. Scelfo RA, Schwielenbercher C, Veronese A, Gramantieri L, Bolondi L, Querzoli P, Nenci I, Calin GA, Angioni A, Barbanti-Brodano G, Negrini M. Loss of methylation at chromosome 11p15.5 is common in human adult tumors. Oncogene. 2002;21:2564–72.

41. Schwielenbercher C, Gramantieri L, Scelfo R, Veronese A, Calin GA, Bolondi L, Croce CM, Barbanti-Brodano G, Negrini M. Gain of imprinting at chromosome 11p15: A pathogenetic mechanism identified in human hepatocarcinomas.Proc Natl Acad Sci USA. 2000;97:5445–9.

42. Díaz-Meyer N, Day CD, Khatod K, Maher ER, Cooper W, Reik W, Junien C, Graham G, Algar E, Der Kaloustian VM, Higgins MJ. Silencing of CDKN1C (p57KIP2) is associated with hypomethylation at KvDMR1 in Beckwith-Wiedemann syndrome. J Med Genet. 2003;40:797–801.

43. Soejima H, Nakagawachi T, Zhao W, Hagishimoto K, Urano T, Matsuura K, Kitajima Y, Takeuchi M, Nakayama M, Oshima M, Miyazaki K, Jho H, Murakami T. Silencing of imprinted CDKN1C gene expression is associated with loss of CpG and histone H3 lysine 9 methylation at DMR-LIT1 in esophageal cancer. Oncogene. 2004;23:4380–8.

44. Fornari F, Gramantieri L, Ferracin M, Veronese A, Sabbage S, Calin GA, Grazi GL, Giovannini C, Croce CM, Bolondi L, Negrini M. MiR-211 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. Oncogene. 2008;27:5651–61.

45. Medina R, Zaidi SK, Liu CG, Stein JL, van Wijnen AJ, Croce CM, Stein GS. MicroRNAs 221 and 222 bypass quiescence and compromise cell survival. Cancer Res. 2008;68:2773–80.

46. Le Sage C, Nagel R, Egan DA, Schrier M, Mesman E, Mangiola A, Anile C, Maira G, Mercatelli N, Ciafre SA, Farace MG, Agami R. Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. EMBO J. 2007;26:3699–708.

47. Matsuda Y, Ichida T, p16 and p27 are functionally correlated during the progression of hepatocarcinogenesis. Mol Biol Med. 2006;39:169–75.

48. Fabregat I, Roncero C, Fernandez M. Survival and apoptosis: a dysregulated balance in liver cancer. Liver Int. 2007;27:155–62.

49. Schattenberg JM, Galle PR, Schuchmann M. Apoptosis in liver disease. Liver Int. 2006;26:904–11.

50. Charlotte F, L’Hermine A, Martin N, Geley Y, Nollet M, Gaulard P, Zafrani B, Kew M, Wands J, Ozturk M. Expression and role of Hepatocyte growth factor/scatter factor-induced intracellular signalling. Int J Exp Pathol. 2000;81:17–30.

51. Lee KH, Choi EY, Hyun MS, Jang BJ, Kim TN, Lee HJ, Eun JY, Kim HG, Yin SS, Lee DS, Kim JH, Kim JR. Role of hepatocyte growth factor/c-Met signaling in regu-lating urokinase plasminogen activator on invasiveness in human hepatocellular carcinoma: a potential therapeutic target. Clin Exp Metastasis. 2008;25:89–96.

52. Bapon C, Patel T. MicroRNA expression profiling: a molecular tool for defining the phenotype of hepatocellular tumors. Hepatology. 2008;47:1807–9.

53. Huang YS, Dai Y, Yu XF, Bao SY, Yin YB, Tang M, Hu CX. Microarray analysis of microRNA expression in hepatocellular carcinoma and non-tumorous tissues without viral hepatitis. J Gastroenterol Hepatol. 2008;23:87–94.

54. Ladeiro Y, Coughy G, Balabaud C, Bioulac-Sage P, Pelletier L, Rebouissou S, Zucman-Rossi J. MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. Hepatology. 2008;47:1955–63.

55. Varnholt H, Drebuer U, Schulze F, Wedemeyer I, Schirmacher P, Dienes HP, Odenthal H. MicroRNA gene expression profile of hepatitis C virus-associated
hepatocellular carcinoma. *Hepatology*. 2008; 47: 1223–32.

68. Wang Y, Lee AT, Ma JZ, Wang J, Ren J, Yang Y, Tantoso E, Li KB, Ooi LL, Tan P, Lee CG. Profiling microRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-5 as a microRNA-224-specific target. *J Biol Chem*. 2008; 283: 13205–13.

69. Wong QW, Lung RW, Law PT, Lai PB, Chan KY, To KF, Wong N. MicroRNA-23a is commonly repressed in hepatocellular carcinoma and potentiates expression of Stathmin1. *Gastroenterology*. 2008; 135: 257–69.

70. Murakami Y, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, Shimotohno K. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene*. 2008; 25: 2357–45.

71. Kutay H, Bai S, Datta J, Motivala T, Pogribny I, Frankel W, Jacob ST, Ghoshal K. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. *J Cell Biochem*. 2006; 99: 671–8.

72. Budhu A, Jia HL, Forgues M, Liu CG, Goldstein D, Lam A, Zanielli KA, Ye QH, Qin LX, Croce CM, Tang ZY, Wang XW. Identification of metastasis-related microRNAs in hepatocellular carcinoma. *Hepatology*. 2008; 47: 957–967.

73. Gramantieri L, Ferracin M, Forini F, Veronese A, Sabioni S, Liu CG, Califà GA, Giovannianni C, Ferrari E, Gráli, Croce CM, Bolondi L, Negrini M. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res*. 2007; 67: 6902–9.

74. Huang Q, Gumireddy K, Schier M, Le Sage C, Nagel R, Sengupta P, Egan DA, Li A, Huang G, Klein-Szanto AJ, Gimotty PA, Katsaras D, Coukos G, Zhang L, Pure E, Agami R. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. *Nat Cell Biol*. 2008; 10: 202–10.

75. Voorhoeve PM, Le Sage C, Schier M, Gillis AJ, Stoop H, Nagel R, Liu YP, van Duin JP, Drost J, Griekspoor A, Zieliyonski Y, Yabuta N, De Vlta G, Nojima H, Looijenga LH, Agami R. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell*. 2006; 124: 1169–81.

76. Ako Y, Nakagawa Y, Naoe T, Iwai-7 microRNA functions as a potential growth suppressor in human colon cancer cells. *Bio Pharm Bull*. 2006; 29: 903–6.

77. Bussing I, Slack FJ, Grosshans H, let-7 microRNAs in development, stem cells and cancer. *Trends Mol Med*. 2008; 14: 400–9.

78. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. *Cell*. 2005; 120: 635–47.

79. Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. *Genes Dev*. 2007; 21: 1025–30.

80. Mayer C, Hemmann MT, Bartel DP. Disrupting the pairing between let-7 and Hmgα2 enhances oncogenic transformation. *Science*. 2007; 315: 1576–9.

81. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res*. 2004; 64: 3753–6.

82. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E. let-7 regulates self renewal and tumorigenesis of breast cancer cells. *Cell*. 2007; 131: 1109–23.

83. Esquela-Kerscher A, Slack FJ. Oncormirs – microRNAs with a role in cancer. *Nat Rev Cancer*. 2006; 6: 257–69.

84. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer*. 2006; 6: 857–66.

85. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000; 100: 57–70.

86. Datta J, Kutay H, Nasser MW, Nuovo GJ, Wang B, Majumder S, Liu CG, Velina S, Croce CM, Schmittgen TD, Ghoshal K, Jacob ST. Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. *Cancer Res*. 2008; 68: 5049–58.

87. Kim S, Lee UJ, Kim MN, Lee EJ, Kim JY, Lee MY, Choug S, Kim YJ, Choi YC. MicroRNA miR-199a regulates the MET proto-oncogene and the downstream extracellular signal-regulated kinase 2 (ERK2). *J Biol Chem*. 2008; 283: 18158–66.

88. Schulte JH, Horn S, Otto T, Samans B, Heukamp LC, Eilers UC, Krause M, Astrahantseff K, Klein-Hitpass L, Buettner R, Schramm A, Christiansen H, Eilers M, Eggert A, Bervanger B. MYCN regulates oncogenic MicroRNAs in neuroblastoma. *Int J Cancer*. 2008; 122: 699–704.

89. Tarasov V, Jung P, Verdoold B, Lodigyn D, Epanchina T, Pregnancy and Neisseria, Meneghietti G, Heremans J, Differentiation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. *Cell Cycle*. 2007; 6: 1586–93.

90. Cifare SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, Negrini M, Maira G, Croce CM, Farace MG. Extensive modulation of a set of microRNAs in primary glioblastomas. *Biochem Biophys Res Commun*. 2005; 334: 1351–8.

91. He H, Pazdur R, Ferracin M, Ferraro A, Berlingerio MT, Troncone G, Chiappetta G, Liu CG, Santoro M, Negrini M, Croce CM, Fusco A. MicroRNA deregulation in human thyroid papillary carcinomas. *Endocr Relat Cancer*. 2006; 13: 497–508.

92. Galardi S, Mercatelli N, Giorda E, Massalini S, Frajese GV, Cifare SA, Farace MG, miR-211 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27kip1. *J Biol Chem*. 2007; 282: 23716–24.

93. Gottardo F, Liu CG, Ferracin M, Calin GA, Fassan M, Bassi P, Sevignani C, Byrne D, Negrini M, Pagano F, Gomella LG, Croce CM, Baffa R. MicroRNA profiling in kidney and bladder cancers. *Urol Oncol*. 2007; 25: 387–92.

94. Schmelzle T, Mailleux AA, Overholtzer M, Carroll JS, Solimini NL, Lightcap ES, Veiby OP, Brugge JS. Anoikis resistance and tumor metastasis. *Cell Cycle*. 2007; 6: 1586–93.

95. Meng F, Henson R, Wehe-Janh K, Ghoshal K, Jacob ST, Patel T, MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology*. 2007; 133: 647–58.

96. Asangani IA, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S, Allgayer H. MicroRNA-21 (miR-21) post-
transcriptionally downregulates tumor suppressor Ptdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene. 2008; 27: 2128–36.

Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem. 2008; 283: 1026–33.

Zhu S, Si ML, Wu H, Mu YY. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). J Biol Chem. 2007; 282: 14328–36.

Zauberman A, Lupo A, Oren M. Identification of p53 target genes through immune selection of genomic DNA: the cyclin G gene contains two distinct p53 binding sites. Oncogene. 1995; 10: 2361–6.

Okamoto K, Beach D. Cyclin G is a transcriptional target of the p53 tumor suppressor protein. EMBO J. 1994; 13: 4816–22.

Endo Y, Fujita T, Tamura K, Tsuruga H, Nojima H. Structure and chromosomal assignment of the human cyclin G gene. Genomics. 1996; 38: 92–5.

Jensen MR, Factor VM, Zimonicje DB, Miller MJ, Keck CL, Thorgerisson SS. Chromosome localization and structure of the murine cyclin G1 gene promoter sequence. Genomics. 1997; 45: 297–303.

Okamoto K, Li H, Jensen MR, Zhang T, Tay Y, Thorgerisson SS, Prives C. Cyclin G recruits PP2A to dephosphorylate Mdm2. Mol Cell. 2002; 9: 761–71.

Ohtsuka T, Jensen MR, Kim HG, Kim KT, Lee SW. The negative role of cyclin G in ATM-dependent p53 activation. Oncogene. 2004; 23: 5405–8.

Kampmeier J, Behrens A, Wang Y, Yee A, Anderson WF, Hall FL, Gordon EM, McDonnell PJ. Inhibition of rabbit keratocyte and human fetal lens epithelial cell proliferation by retrovirus-mediated transfer of antisense cyclin G1 and antisense MAT1 constructs. Hum Gene Ther. 2000; 11: 1–8.

Gordon EM, Liu PX, Chen ZH, Liu L, Whitley MD, Gee C, Groshen S, Hinton DR, Beart RW, Hall FL. Inhibition of metastatic tumor growth in nude mice by portal vein infusions of matrix-targeted retroviral vectors bearing a cytotoxic cyclin G1 construct. Cancer Res. 2000; 60: 3343–7.

Skotko M, Wu L, Anderson WF, Gordon EM, Hall FL. Retroviral vector-mediated gene transfer of antisense cyclin G1 (CYCG1) inhibits proliferation of human osteogenic sarcoma cells. Cancer Res. 1995; 55: 5493–8.

Jensen MR, Factor VM, Fantozzi A, Helin K, Huh CG, Thorgerisson SS. Reduced hepatic tumor incidence in cyclin G1-deficient mice. Hepatology. 2003; 37: 862–70.

Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol. 2001; 35: 421–30.

Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y, Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer. 1985; 56: 918–28.

Liver Cancer Study Group of Japan. Predictive factors for long term prognosis after partial hepatectomy for patients with hepatocellular carcinoma in Japan. Cancer. 1994; 74: 2772–80.

Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. Identification of p57(KIP2) as a new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Jpn J Clin Oncol. 1999; 31: 133–41.

The Cancer of the Liver Italian Program (CLIP). Prospective validation of the CLIP score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. Hepatology; 2000; 31: 840–5.

Leung TW, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, Lau JT, Yu SC, Johnson PJ. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: a study based on 926 patients. Cancer. 2002; 94: 1760–9.

Barbara L, Benzi G, Gaiani S, Fusconi F, Zironi G, Siringo S, Rigamonti A, Barbara C, Grigioni W, Mazzotti A, Bolondi L. Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. Hepatology. 1992; 16: 132–7.

Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular carcinoma. Cancer. 2004; 5: 215–9.

Kaposi-Novak P, Lee JS, Gomez-Quiroz L, Coulouarn C, Factor VM, Thorgerisson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. J Clin Invest. 2006; 116: 1582–95.

Ito Y, Matsura N, Sakon M, Miyoshi E, Noda K, Takeda T, Umeshiba K, Nagano H, Nakamori S, Dono K, Tsujimoto M, Nakahara M, Nakao K, Taniguchi N, Monden M. Expression and prognostic roles of the G1-S modulators in hepatocellular carcinoma: p27 independently predicts the recurrence. Hepatology. 1999; 30: 90–5.

Tannapfel A, Grund D, Katalinic A, Uhlmann D, Kocherling U, Wasner M, Hauss J, Engelkamp R, Wittekind C. Decreased expression of p27 protein is associated with advanced tumor stage in hepatocellular carcinoma. Int J Cancer. 2000; 89: 350–5.

Nakai S, Masaki T, Shiratori Y, Ohgi T, Morishita A, Kurokohchi K, Watanabe S, Kuriyama S. Expression of p57(KIP2) in hepatocellular carcinomas: relationship between tumor differentiation and patient survival. Int J Oncol. 2002; 20: 769–75.

Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, Tsunoda T, Furukawa Y, Nakamura Y. Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. Cancer Res. 2001; 61: 2129–37.

Delpuech O, Trabut JB, Carnot F, Feuillard J, Brechet C, Kremsdorf D. Identification, using cDNA macroarray analysis, of distinct gene expression profiles associated with pathological and virological features of hepatocellular carcinoma. Oncogene. 2002; 21: 2926–37.

Monden M. Clinical management of hepatocellular carcinoma: a new prognostic system for predicting the natural history of hepatocellular carcinoma in Japan. Cancer. 1985; 56: 918–28.

Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y, Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer. 1985; 56: 918–28.

Chevret S, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. Identification of p57(KIP2) as a new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Jpn J Clin Oncol. 1999; 31: 133–41.

The Cancer of the Liver Italian Program (CLIP). Prospective validation of the CLIP score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. Hepatology; 2000; 31: 840–5.

Leung TW, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, Lau JT, Yu SC, Johnson PJ. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program stagingsystem: a study based on 926 patients. Cancer. 2002; 94: 1760–9.

Barbara L, Benzi G, Gaiani S, Fusconi F, Zironi G, Siringo S, Rigamonti A, Barbara C, Grigioni W, Mazzotti A, Bolondi L. Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. Hepatology. 1992; 16: 132–7.

Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular carcinoma. Cancer. 2004; 5: 215–9.

Kaposi-Novak P, Lee JS, Gomez-Quiroz L, Coulouarn C, Factor VM, Thorgerisson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. J Clin Invest. 2006; 116: 1582–95.
127. Ye GH, Qin LX, Forgue M, He P, Kim JW, Peng AC, Simon R, Li Y, Robles AI, Chen Y, Ma ZC, Wu ZQ, Ye SL, Liu YK, Tang ZY, Wang XW. Predicting hepatitis B virus-positive metastatic hepatocellular carcinoma using gene expression profiling and supervised machine learning. *Nat Med.* 2003; 9: 416–23.

128. Wang SM, Ooi LL, Hui KM. Identification and validation of a novel gene signature associated with the recurrence of human hepatocellular carcinoma. *Clin Cancer Res.* 2007; 13: 6279–83.

129. Iizuka N, Oka M, Yamada-Okabe H, Nishida M, Maeda Y, Mori N, Takao T, Tamesa T, Tangoku A, Tabuchi H, Hamada K, Nakayama H, Ishitsuka H, Miyamoto T, Hiramatsu Y, Uchimura S, Hamada K, Nakayama H, Ishitsuka H, Tamesa T, Tangoku A, Tanaka T, Calvin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell.* 2006; 9: 189–98.

130. Kurokawa Y, Matoba R, Takemasa I, Nagano H, Dono K, Nakamori S, Iizuka N, Oka M, Yamada-Okabe H, Wang XW. A microRNA expression signature of liver cirrhosis. *Anticancer Drugs.* 2004; 15: 282: 1479–86.

131. Lee JS, Park BJ, Kim KS, Park SC, Chung YJ, Kim BG, Yoon JH, Lee HS, Kim CY, Noh S, Suh KS, Lee KU, Chu IS, Raskams T, Thorgeirsson SS, Kim YJ. Gene expression-based recurrence prediction of hepatocellular carcinoma. *J Hepatol.* 2004; 41: 284–91.

132. Woo HG, Park ES, Cheon JH, Kim JO, Lee JS, Park BJ, Kim KS, Park SC, Chung YJ, Kim BG, Yoon JH, Lee HS, Kim CY, Noh S, Suh KS, Lee KU, Chu IS, Raskams T, Thorgeirsson SS, Kim YJ. Gene expression-based recurrence prediction of hepatitis B virus-related hepatocellular carcinoma. *Clin Cancer Res.* 2008; 14: 2056–64.

133. Lee JD, Yun M, Lee JM, Choi Y, Choi YH, Kim JS, Kim SJ, Kim KS, Yang WI, Park YN, Han KH, Lee WJ, Yoo N, Lim SM, Park JH. Analysis of gene expression profiles of hepatocellular carcinomas with regard to 18F-fluorodeoxyglucose uptake pattern on positron emission tomography. *Eur J Nucl Med Mol Imaging.* 2004; 31: 1521–30.

134. Kulshreshtha R, Ferracin M, Wojciech SE, Garzon R, Alder H, Agosto-Perez FJ, Davuluri R, Liu CG, Croce CM, Negrini M, Calvin GA, Ivan M. A microRNA signature of hypoxia. *Mol Cell Biol.* 2007; 27: 1859–67.

135. Camps C, Bufla FM, Coletta S, Moore J, Sotiriou C, Sheldon H, Harris AL, Gleadle JM, Ragoussis J. hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in breast cancer. *Clin Cancer Res.* 2008; 14; 1340–8.

136. Volinia S, Calvin GA, Liu CG, Amba S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scapra A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA.* 2006; 103: 2257–61.

137. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, YI M, Stephens RM, Okamoto A, Yokota T, Tanaka T, Calvin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell.* 2006; 9: 189–98.

138. Iorio MV, Ferracin M, Liu CG, Veronesi A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calvin GA, Querzoli P, Negrini M, Croce CM. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res.* 2005; 65: 7605–70.

139. Korka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL, Visakorpi T, Porkka KP, Pfeiffer MJ, Waltering KK, Negrini M, Harris CC, Croce CM. MicroRNA expression profiling in prostate cancer. *Cancer Res.* 2007; 67: 6130–5.

140. Salvi A, Arici B, Portolani N, Guilini SM, De Petro G, Barta AL. *In vivo* c-met inhibition by antisense RNA and plasmid-based RNAi down-modulates migration and invasion of hepatocellular carcinoma cells. *Int J Oncol.* 2007; 31: 451–60.

141. Treibel G, Wex T, Schleyer E, Troeger U, Hosius C, Maltertheiner P. Imatinib for hepatocellular carcinoma–focus on pharmacokinetic/pharmacodynamic modelling and liver function. *Cancer Lett.* 2008; 260: 146–54.

142. Hopfner M, Schuppan D, Scherubl H. Growth factor receptors and related signalling pathways as targets for novel treatment strategies of hepatocellular cancer. *World J Gastroenterol.* 2008; 14: 1–14.

143. Stock P, Monga D, Tan X, Micsenyi A, Loizos N, Monga SP. Platelet-derived growth factor receptor-alpha: a novel therapeutic target in human hepatocellular cancer. *Mol Cancer Ther.* 2007; 6: 1932–41.

144. Ramadori G, Fuzesi L, Grabe B, Piefer T, Armbrust T. Successful treatment of hepatocellular carcinoma with the tyrosine kinase inhibitor imatinib in a patient with liver cirrhosis. *Anticancer Drugs.* 2004; 15: 405–9.

145. Eckel F, von Delius S, Mayr M, Dobritz M, Feld F, Hosius C, Schleyer E, Schulte-Frohlinde E, Schmid RM, Lersch C. Pharmacokinetic and clinical phase II trial of imatinib in patients with impaired liver function and advanced hepatocellular carcinoma. *Oncology.* 2005; 69: 363–71.

146. Lin AY, Fisher GA, So S, Tang C, Levitt L. Phase II study of imatinib in unresectable hepatocellular carcinoma. *Am J Clin Oncol.* 2008; 31: 84–8.

147. Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, Wilheim S, Lynch M, Carter C. Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. *Cancer Res.* 2006; 66: 11851–8.

148. Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol.* 2008; 48 Suppl 1: S20–37.

149. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raouf JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Garet TF, Galle PR, Seitz JF, Borbath I, Haussinger D, Giannaris T, Shan M, Moscovici M, Violitsis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med.* 2008; 359: 378–80.

150. Song E, Lee SK, Wang J, Ince N, Ouyang N, Min J, Chen J, Shankar P, Lieberman J. RNA interference targeting Fas protects mice from fulminant hepatitis. *Nat Med.* 2003; 9: 347–51.

151. Cimmino A, Calvin GA, Fabbrini M, Iorio MV, Ferracin M, Shimizu M, Wojciech SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA.* 2005; 102: 7797–802.

152. Song E, Lee SK, Wang J, Ince N, Ouyang N, Min J, Chen J, Shankar P, Lieberman J. RNA interference targeting Fas protects mice from fulminant hepatitis. *Nat Med.* 2003; 9: 347–51.

153. Cimmino A, Calvin GA, Fabbrini M, Iorio MV, Ferracin M, Shimizu M, Wojciech SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA.* 2005; 102: 13944–9.

154. Scott KG, Goga A, Bhaumik D, Berger CE, Sullivan CS, Benz GC. Coordinate Suppression of ERBB2 and ERBB3 by Enforced Expression of Micro-RNA miR-125a or miR-125b. *J Biol Chem.* 2007; 282: 1479–86.

155. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res.* 2005; 65: 6029–33.
154. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. Oncogene. 2007; 26: 2799–803.

155. Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, Jiang J, Schmittgen TD, Patel T. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology. 2006; 130: 2113–29.

156. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with ‘antagomirs’. Nature. 2005; 438: 685–9.

157. Elmen J, Lindow M, Schutt S, Lawrence M, Petri A, Obad S, Lindholm M, Hedtjarn M, Hansen HF, Berger U, Gullans S, Kearney P, Sarnow P, Staarup EM, Kauppinen S. LNA-mediated microRNA silencing in non-human primates. Nature. 2008; 452: 896–9.

158. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med. 1996; 334: 693–9.

159. Jiang J, Gusev Y, Aderca I, Mettler TA, Roberts LR, Schmittgen TD. Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. Clin Cancer Res. 2008; 14: 419–27.

160. Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, Kim JW, Kim S. MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res. 2008; 14: 2690–5.

161. Prueitt RL, Yi M, Hudson RS, Wallace TA, Howe TM, Yantis HG, Lee DH, Stephens RM, Liu CG, Calin GA, Croce CM, Amb S. Expression of microRNAs and protein-coding genes associated with perineural invasion in prostate cancer. Prostate. 2008; 68: 1152–64.

162. Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE. MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metab. 2008; 93: 1600–8.

163. Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu CG, Alder H, Calin GA, Menard S, Croce CM. MicroRNA signatures in human ovarian cancer. Cancer Res. 2007; 67: 8699–707.