[Frontiers in Bioscience, Landmark, 20, 78-104, January 1, 2015]

The role of circulating microRNA in hepatocellular carcinoma

Simon Chang-Hao Tsao[1,2], Andreas Behren[2], Jonathan Cebon[2], Christopher Christophi[1]

[1]The University of Melbourne, Department of Surgery Austin Health, Studley Road, Heidelberg, Victoria, Australia 3084, [2]The Ludwig Institute for Cancer Research, Level 5, Olivia Newton-John Cancer and Wellness Centre, Austin Health, 145-163 Studley Road, Heidelberg, Victoria, Australia 3084

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Search Strategy
4. Circulating miRNA
- *4.1. miR-18a*
  - *4.1.1. Tissue*
  - *4.1.2. Serum*
- *4.2. miR-92a*
  - *4.2.1. Tissue*
  - *4.2.2. Serum*
- *4.3. miR-26a*
  - *4.3.1. Tissue*
  - *4.3.2. Serum*
- *4.4. miR-221*
  - *4.4.1. Tissue*
  - *4.4.2. Serum*
- *4.5. miR-224*
  - *4.5.1. Tissue*
  - *4.5.2. Serum*
- *4.6. miR-500*
  - *4.6.1. Tissue*
  - *4.6.2. Serum*
- *4.7. miR-885-5p*
  - *4.7.1. Tissue*
  - *4.7.2. Serum*
- *4.8. miR-15b, -130b*
  - *4.8.1. Tissue*
  - *4.8.2. Serum*
- *4.9. miR-21*
  - *4.9.1. Tissue*
  - *4.9.2. Serum*
- *4.10. miR-223*
  - *4.10.1. Tissue*
  - *4.10.2. Serum*
- *4.11. miR-122*
  - *4.11.1. Tissue*
  - *4.11.2. Serum*
- *4.12. miR-16*
  - *4.12.1. Tissue*
  - *4.12.2. Serum*

4.13. miR-195, -199a
4.13.1. Tissue
4.13.2. Serum
4.14. miR-21, -26a, -27a, -122, -192, -223, -801
4.14.1. Tissue
4.14.2. Serum
4.15. miR-25, -375, let-7f
4.15.1. Tissue
4.15.2. Serum
5. Discussion
6. Acknowledgement
7. References

1. ABSTRACT

Hepatocellular carcinoma (HCC) is a rapidly progressing disease that exerts a huge burden on patients and health care systems. Rapid progression and difficulty in detecting early disease are major obstacles in offering potentially curative treatments. Besides the lack of effective chemo- or immunotherapy for advanced disease, there are currently no reliable tumor markers or imaging technologies that can accurately diagnose early HCC or predict disease progression. Since the discovery of microRNA (miRNA) and its involvement in hepatocarcinogenesis, the literature describes their usefulness as potential new biomarkers and treatment targets. Some of these miRNAs can also be found in the systemic circulation. With advances in detection and sequencing technologies, an increasing amount of data demonstrate the possibility of using circulating miRNAs as biomarkers to improve our current management of HCC in a less-invasive manner. This paper will review circulating miRNAs with a known function in HCC, describing their role and function in tumorigenesis. This review discusses their potential use as biomarkers in conjunction with emerging treatments in the diagnosis and targeting of this disease.

2. INTRODUCTION

Liver cancer is the 5th most frequently diagnosed cancer in men worldwide and the second most frequent cause of cancer death. Jemal *et al.* (2011) in their analysis of global cancer statistics reported an estimated 748,300 new liver cancer cases and 695,900 liver cancer related deaths worldwide in 2008. (1) Among primary liver cancers, hepatocellular carcinoma (HCC) represents the major histological subtype, accounting for 70% to 85% of the total liver cancer burden worldwide, with a median survival following diagnosis of just 6-20 months. (2, 3)

The poor outcome can be attributed to late diagnosis and the aggressive nature of the disease. HCC is characterized by progressive development and a high postsurgical recurrence rate (up to 40% within the first year and 54% at 5 years). (4-8) Currently, resection or liver transplantation are the best options for a potential cure for HCC. Importantly, the overall survival rate at 5 years after liver transplantation is approximately 70-75% for a sub-set of patients based on clinical staging and 40%-65% for liver resection. (9) For patients who are not eligible for surgical treatment (>80%), the 5 year survival drops below 10%. (4)

HCC is currently diagnosed based on a combination of radiological criteria and serum alpha-fetoprotein (AFP) level and only rarely with histological biopsies. (10) AFP is a glycoprotein, normally produced by the fetal liver and yolk sac during gestation, the level normalises to 10-20μg/L by 12 months of age. Raised levels of AFP (100-4000 μg/L depending on gender) may indicate HCC, but it may also be associated with liver injury and other malignancies, such as gastric cancer and tumors of gonadal origin (both germ and non-germ cell). (11-14) AFP remains the main tumor marker used today

despite having been discarded by the American Association for the Study of Liver Disease for the surveillance and diagnosis of HCC in their practice guidelines (AASLD). (15)

Numerous serum tumor markers have been proposed over the last decade in the hope to increase diagnostic accuracy. These include DCP, AFP-L3, VEGF and IGF-1. (16, 17) This is a reflection of the heterogeneous nature of the HCC tumors as a result of their diverse aetiology, which leads to wide variability of marker expressions. (18, 19) Furthermore, independently arising HCCs within the same patient can also show distinctive gene expression. (20) There is a clear need of a novel tumor marker which can assist with diagnosis, prognosis and treatment.

MicroRNA (miRNA) is a group of small non-coding RNAs, approximately 22 nucleotides in length that can accurately regulate gene expression by complementary base pairing with the target messenger RNAs (mRNAs). (21) Since their discovery in nematode *C. elegans* in 1993, (22) significant research has led to a better understanding of their function. It has been estimated by computational analysis that over 50% of human protein-coding genes are regulated by miRNAs and that each miRNA may target hundreds of different targets. (23) Some miRNAs can work together as a cluster to accomplish a common function, they often have a similar sequence and each cluster usually consists of 2 to 3 (but may exceed 8) members. (24) Through their ability to regulate gene expression, miRNA can participate not only in normal cell homeostasis and development processes such as cell cycle, cell growth, proliferation and apoptosis, but can also act as tumor suppressors and oncogenes, controlling tumor invasion, metastasis, and drug resistance. (25, 26) Dysregulated miRNA with oncogenic potential is often termed Oncomir. (27)

Numerous articles describe the association of miRNA with hepatocarcinogenesis and their use as potential markers for all aspects of HCC management including diagnosis, differentiating etiology, monitoring disease progression, treatment response, prognosis and as novel therapeutic targets. (28, 29) This is summarized in Table 1. Although most of the information came from HCC tissue studies, increasing evidence suggests alterations were also reflected in patients' serum miRNA level (30). Enumerating circulating miRNA would allow for the possibility of gathering information during treatment planning prior to surgery, which would make circulating miRNA a novel marker that can improve our current HCC management. This review will focus on this subgroup of miRNAs.

3. SEARCH STRATEGY

The literature search involved all relevant published peer reviewed articles from PubMed, Ovid, and Medline in English language until July 2013. The search terms were combinations of "hepatocellular carcinoma/ hepatoma/liver cancer/liver neoplasm" plus one of the following "microRNA", "serum microRNA", "circulating microRNA", "diagnosis", "prognosis" and "recurrence". Only articles that have discussed the use of miRNA as markers of HCC were included. We subsequently found 104 articles that fit these criteria.

4. CIRCULATING MIRNA

Circulating miRNA in liver disease was first demonstrated by Wang *et al.*, using an acetaminophen overdose-induced liver injury mouse model. The authors found a group of miRNAs (e.g. miR-122, miR-192 among many others) that showed dose- and exposure duration-dependent changes in their plasma level, which paralleled changes in serum aminotransferases (AST) level. (31) Zhang *et al.*, subsequently found that circulating miR-122 would rise before AST in patients developing liver injury from HBV infection and furthermore, circulating miR-122 level was disease severity-dependent and correlated with histological stages. (32)

MiRNA is remarkably stable in the systemic circulation as they can avoid ribonucleases by forming lipid or lipoprotein

Table 1. Summary of aberrantly expressed circulating miRNA in HCC

miRNA	Expression changes (reference)	Study sample	Significance
miR-1	Up (51)	50 Healthy Liver (HL) 55 HBV-HCC	Hepatitis B related HCC (HBV-HCC) Significantly upregulated from control. (p<0.0001)
miR-15b	Up (91)	30 HL 153 HCC (141 HBV) 30 HBV carriers	Distinguish HCC from NC with a sensitivity of 98.3%, specificity of 15.3% and AUC[1] of 0.485. Level decrease post resection P=0.0637
miR-16	Down (120)	71 HL, 105 HCC (20 HBV, 66 HCV), 107 Chronic liver disease (CLD) (8 HBV, 59 HCV)	Significant association with HCC (HCC versus NC, HCC versus CLD, CLD versusNC, all P<0.01), combine with AFP improves accuracy.
miR-18a	Up (50)	60 HL 101 HBV-HCC 30 HBV chronic hepatitis or cirrhosis	Discriminate HBV- HCC from healthy controls with AUC 0.881, sensitivity 86.1%, specificity 75%. HBV-HCC from HBV cirrhosis or chronic hepatitis AUC 0.775, sensitivity 77.2%, specificity 70%
miR-21	Up (105)	89 HL, 101 HCC (76 HBV-HCC), 48 CH (all HBV+ve)	Significantly elevated in HCC (p<0.0001)
miR-21	Up (86)	20 HL, 46 HCC (30 HBV-HCC)	Elevated in HCC
miR-21	Up (70)	68 HL 204 HBV-HCC 75 Chronic HBV 60 HBV-Cirrhosis	Significantly upregulated HBV-HCC versus Healthy+CBH+HBV-Cirrhotic group. P<0.001 AUC to distinguish HBV-HCC from all those other groups combined is 0.626
miR-21	Up (94)	30 HL 29 HBV 57 HBV-HCC	Distinguish HBV-HCC from healthy+HBV group with sensitivity 89.47%, specificity 71.19%, AUC 0.865 Level decrease post resection (p=0.0648)
miR-25	Up (51)	50 HL 55 HBV-HCC	Significantly upregulated from control. (p<0.0001) When miR-25 and let-7f were tested together with miR-375, it increased the AUC to 0.9967+/- 0.015 (specificity 99.1%; sensitivity 99.1%)
miR-26a	Down (70)	68 HL 204 HBV-HCC 75 Chronic HBV 60 HBV-Cirrhosis	Significantly downregulated when compared to Healthy+CBH+HBV-Cirrhotic group. P<0.001 AUC to distinguish HBV-HCC from all those other groups combined is 0.665
miR-27a	Down (70)	68 HL 204 HBV-HCC 75 Chronic HBV 60 HBV-Cirrhosis	Significantly downregulated when compared to Healthy+CBH+HBV-Cirrhotic group. P<0.001 AUC to distinguish HBV-HCC from all those other groups combined is 0.638
miR-92a	Down (52)	10 HL, 10 HCC (3HBV, 5 HCV, 2 non-b, non-c)	Decreased compare to HL (p=0.022) Decreases post-resection (p=0.082)
miR-92a	Up (51)	50 HL 55 HBV-HCC	Significantly elevated in HBV-HCC P<0.0001
miR-122	Up (105)	89 HL, 101 HCC (76 HBV-HCC), 48 CH (all HBV+ve)	Significantly elevated in HCC. P<0.0001 AUC 0.93 to diagnose CH from control, AUC 0.79 to diagnose HBV-HCC from CH, if other cause for liver injury has been excluded.

(Contd...)

Table 1. Contd...

miRNA	Expression changes (reference)	Study sample	Significance
miR-122	Up (115)	24 HL 38 Chronic HCV with normal ALT 64 Chronic HCV with elevated ALT	Significantly upregulated in chronic HCV hepatitis (p<0.001) Discrimination chronic HCV infection from HL (p=0.026) AUC 0.97
miR-122	Down (70)	68 HL 204 HBV-HCC 75 Chronic HBV 60 HBV-Cirrhosis	Significantly downregulated when compared to Healthy+CBH+HBV-Cirrhotic group. P<0.062 AUC to distinguish HBV-HCC from all those other groups combined is 0.553
miR-122	Down (87)	85HL 85 HCC (75 are HBV-HCC)	Significantly upregulated compare to healthy. P<0.001 Diagnose HCC, AUC O.707, sensitivity 70.6%, specificity 67.1%. (no superior to AFP) When combined with AFP, AUC 0.943, sensitivity 87.1%, specificity 98.8%
miR-130b	Up (94)	30 HL 153 HCC patients (141 HBV+ve) 30 HBV carriers	Distinguish HCC from healthy AUC 0.913, sensitivity of 87.7%, specificity of 81.4%. When combined with miR-15b, AUC 0.981, sensitivity of 98.3%, specificity of 91.5%. Level decrease post resection p=0.0158
miR-183	Up (94)	30 HL 153 HCC patients (141 HBV+ve) 30 HBV carriers	Significantly elevated in HBV-HCC. Distinguish HBV-HCC from healthy+HBV group with sensitivity 57.89%, specificity 69.49%, AUC 0.661 Level decrease post resection P=0.0084
miR-192	Up (115)	24 HL 38 Chronic HCV with normal ALT 64 Chronic HCV with elevated ALT	Significantly upregulated in chronic HCV hepatitis (p<0.001)
miR-192	Up (70)	68 HL 204 HBV-HCC 75 Chronic HBV 60 HBV-Cirrhosis	Significantly upregulated in HBV-HCC when compared to Healthy+CBH+HBV-Cirrhotic group. P=0.016 Distinguish HBV-HCC from all those groups combined, AUC 0.569
miR-195	Down (120)	71 HL, 105 HCC (20 HBV, 66 HCV), 107 CLD (8 HBV, 59 HCV)	Significantly downregulated in HCC compared to HL (p<0.01) HCC v.s. CLD (p=0.04) CLD v.s. normal (p<0.01)
miR-199a	Down (120)	71 HL, 105 HCC (20HBV, 66HCV), 107 CLD (8HBV, 59HCV)	Significantly downregulated in HCC compared to healthy control (p<0.01), HCC v.s. CLD (p<0.01) CLD v.s. normal (p<0.01)
miR-206	Up (51)	50 HL 55 HBV-HCC	Significantly upregulated in HBV-HCC compared to control. (p<0.0001)
miR-221	Up (86)	20 HL, 46 HCC (30 HBV-HCC, 16 others)	Elevated in 35/46 HCC, correlates with HCC stage and prognosis (5 year overall survival rate is significantly lower in patients with high miR-221 expression, P<0.05.)

Contd...

Table 1. Contd...

miRNA	Expression changes (reference)	Study sample	Significance
miR-221	Up (87)	85HL 85 HCC (75 are HBV-HCC)	Level is higher in the HCC group but it is not statistically significant (p=0.225).
miR-222	Up (86)	20 HL, 46 HCC (30 HBV-HCC, 16 others)	Elevated in HCC
miR-223	Up (51)	160 HL 135 HBV (both chronic and asymptomatic) 48 HCV 65 HBV-HCC	Significantly elevated in all HBV related liver groups compared to HL. P<0.0001 HCV v.s. control P=0.0043
miR-223	Up (105)	89 HL, 101 HCC (76 HBV-HCC), 48 CH (all HBV+ve)	Distinguish HCC from HL P<0.0001.
miR-223	Down (70)	68 HL 204 HBV-HCC 75 Chronic HBV 60 HBV-Cirrhosis	Significantly downregulated in HBV-HCC when compared to HL+CBH+HBV-Cirrhotic group. P<0.001 AUC to distinguish HBV-HCC from all those groups combined is 0.643
miR-224	Up (86)	20 HL, 46 HCC (30 HBV-HCC, 16 others)	Elevated in HCC
miR-375	Up (51)	50 HL 55 HBV-HCC	HBV-HCC significantly upregulated from HL (p<0.0001) Distinguish HBV-HCC from HL AUC 0.96 (specificity of 96%; sensitivity of 100%) When miR-25 and let-7f were tested together with miR-375, it increased the AUC to 0.9967+/- 0.015 (specificity 99.1%; sensitivity 99.1%)
miR-500	Up (95)	10 HCC (unspecfied aetiology)	Levels increased in 3/10 HCC patients, significantly reduced within 6 months post-HCC resection.
miR-801	Up (70)	68 HL 204 HBV-HCC 75 Chronic HBV 60 HBV-Cirrhosis	Significantly upregulated when compared to HL+CBH+HBV-Cirrhotic group. P<0.001 AUC to distinguish HBV-HCC from all those other groups combined is 0.629
miR-885 (-5p)	Up (93)	24 HL, 46 HCC (33 HBV-HCC), 26 Liver cirrhosis (LC) (20 HBV), 26 chronic HBV	The data demonstrated that patients with HCC, LC or CHB had significantly (P<0.0001) higher serum levels of miR-885-5p than HL
Let-7f	Up (51)	50 HL 55 HBV-HCC	HBV-HCC significantly upregulated from HL. (p<0.0001) Distinguish HBV-HCC from HL when combined with miR-25 and -375, AUC=0.9967 Specificity 99.1%, sensitivity 97.9%
miR-21, -26a, -27a, -122, -192, -223	Up (-21, -192) Down for the rest (70)	68 HL 204 HBV-HCC 75 Chronic HBV 60 HBV-Cirrhosis	Panel consists of miR-21, -26a, -27a, -122, -192, -223, can diagnose HBV-HCC with an AUC of 0.888, regardless of the BCLC stages. Can also differentiate HBV-HCC from HL (AUC=0.941), chronic hepatitis B (AUC=0.842), and cirrhosis (AUC=0.884).

[1]AUC: area under the operating characteristic curve, where 1 is a perfect test.

complexes (such as apoptotic bodies, microvesicles, and exosomes), these complexes can withstand extreme pH and temperature, remaining relatively stable for detection *ex vivo* (i.e. formalin fixed tissue). (30, 33, 34) Circulating miRNA can be detected with RT-PCR, microarray and next-generation sequencing. RT-PCR is the most common technique that utilizes a stem-loop primer binding to the mature miRNA during the reverse transcription step to amplify the desired miRNA. Chen *et al.*, found that miRNA RT-PCR can distinguish single nucleotide differences between related miRNAs. (35) The stability of circulating miRNA allows reliable enumeration, which is vital as a novel diagnostic biomarker.

Study of the circulating miRNA is not complete without understanding their role in liver tissue. There are numerous studies which have attempted to correlate aberrant miRNA expression with various roles in hepatocarcinogenesis. Many used microarray to compare miRNA expression between HCC tissues to their adjacent normal tissues, any aberrantly expressed miRNAs would be confirmed with qRT-PCR and analysed for statistical significance. Various *in silico* and *in vitro* methods would then be employed to identify possible targets of those miRNAs. As a result, several hundreds of tissue miRNAs and their potential targets have been shown to play a role in hepatocarcinogenesis. (36, 37) These tissue study findings will be summarised as we study their corresponding alterations in the circulation (serum) in more than 20 important miRNAs below.

4.1. miR-18a

4.1.1. Tissue

MiR-18a is a member of the miR-17-92 cluster, which consists of miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92-1. This cluster is one of the best characterized oncogenic miRNA clusters and has been designated as Oncomir-1. (27) Over-expression of this cluster has been linked to hepatocarcinogenesis and many other cancers. (27, 38-41) MiR-18a was found to be significantly upregulated in both HCV and HBV-related HCC. (42-45) Connolly *et al.*, demonstrated that by knocking down the miR-17-92 cluster, the HepG2 cell line showed reduction in proliferation and anchorage-independent growth. (43) Wu *et al.*, identified in gastric cancer that miR-18a could suppress PIAS3 (Protein Inhibitor of Activated Signal Transducer and Activator of Transcription 3), which resulted in elevated STAT3 (Signal Transducer and Activator of Transcription 3) levels and their downstream transcriptional targets, namely the inhibitors of apoptosis Survivin, Bcl-xL and c-Myc. (46)

One report demonstrated that the estrogen receptor 1 (ESR1) gene, which encodes estrogen receptor alpha (ERα), was potentially a target of miR-18a as increased miR-18a level was associated with ERα suppression. (45) Given that the miR-18a level was significantly higher in female HCC tissue compared to males, the authors hypothesised that estrogen might have a potential protective role against HCC. (45) However, this does not fully explain why HCC is more prevalent in males given there are other reports demonstrating that estrogen can promote liver regeneration and growth, and has been found to promote hepatocarcinogenesis and tumor progression *in vitro* and in animal models. (47-49)

4.1.2. Serum

Serum miR-18a level was found to be significantly elevated in HBV-related HCC patients when compared to healthy controls and patients with HBV-related chronic hepatitis or cirrhosis. (50) The serum level of miR-18a could be used to discriminate HBV-related HCC from healthy controls with an area under the operating characteristic curve (AUC) of 0.881 with 86.1% sensitivity and 75% specificity. This demonstrated the potential diagnostic role of serum miR-18a in clinical practice. (50)

4.2. miR-92a

4.2.1. Tissue

MiR-92a is also a member of the miR-17-92 family. Connolly *et al.*, (43) and Li *et al.*, (51) showed that miR-92a was significantly overexpressed in HBV-related

HCC tissues compared to HBV-infected liver, or normal adjacent liver tissues. This is supported by Shigoka *et al.*, using *in situ* hybridization on a small group of HCC tumors (45% HCV and 27% HBV-related) and found strong miR-92a staining in most HCC cells with no staining in the surrounding normal tissues. The same group further demonstrated anti-miR-92a antagomir, an engineered oligonucleotide which can block miR-92a, transfected HCC cell lines had a decreased proliferation rate compared to control. (52) Several potential miR-92a targets have been verified or predicted in other tumors such as FAN, DKK3, NOX4 and AURKA, however, more studies are required to assert their roles in hepatocarcinogenesis. (53)

4.2.2. Serum

Li *et al.*, showed that in a group of 55 HBV-related HCC patients, serum miR-92a levels were significantly elevated compared to healthy controls. (50) However, Shigoka's group analysed blood from 10 HCC patients (of unspecified aetiology) and found their circulating miR-92a level to be significantly decreased compared to healthy control, contradicting their own HCC tissue findings. (52) The reason for the discrepancy between the studies is unknown. Studies with large cohorts of patients using a standardised reference are required to evaluate the potential clinical role of serum miR-92a.

4.3. miR-26a

4.3.1. Tissue

MiR-26a is a tumor suppressor and its expression in HCC tissue is found to be significantly downregulated compared to adjacent normal liver. Kota *et al.*, found that miR-26a is able to induce G1 cell arrest by directly suppressing cyclin D2 and cyclin E2 *in vitro*. In a murine HCC model, mice transfected with adeno-associated virus carrying miR-26a showed inhibition of cancer proliferation, induction of tumor-specific apoptosis and a dramatic delay in disease progression. (54)

MiR-26a expression can be an indicator of disease severity with significantly downregulated miR-26a expression found in HCCs with vascular/lymphatic invasion or intrahepatic metastasis. (49, 55) Downregulated miR-26a is also associated with higher TNM stage, higher likelihood of recurrence, metastasis and shorter overall survival. (55, 56)

Many studies suggest miR-26a is involved in complex interactions between estrogen, estrogen-receptor alpha (ERα), interleukin-6 (IL-6) and transcription factor STAT3 (signal transducer and activator of transcription 3) associated gene. (49, 55)

Chen *et al.*, found that oestradiol (E2) promotes HCC tumor growth in a similar way as it promotes breast cancer via the E2 to ERα pathway. (49) MiR-26a is able to significantly downregulate ERα and hence prevents the promoter effect of E2 on HCC cells. Women normally have a significantly higher expression of miR-26a in their hepatocytes, which may explain their significantly better prognosis and lower incidences of HCC compared to men. (57-60)

IL-6 is a multifunctional cytokine largely involved with hepatocyte and systemic inflammation. It has been found to be able to promote tumor growth and invasion in many types of tumors including HCC. (61-66) Serum and tissue levels of IL-6 increase with chronic liver inflammation and HCC, and it is inversely related to miR-26a expression. (55, 67, 68) Yang *et al.*, discovered that IL-6 is a target of miR-26a and knocking down IL-6 or transfecting cells with miR-26a can suppress HCC progression. (56) Interestingly, estrogen can also suppress IL-6 production by Kupffer cells. (65)

IL-6 can also lead to cancer formation through the IL-6-STAT 3 signaling pathway. (69) STAT 3 belongs to the STAT protein family, which is a cytoplasmic protein that can be activated by cytokines and act as a transcription factor in the nucleus to control cell growth and apoptosis. Yang *et al.*, demonstrated that they were able to downregulate many of the IL-6/STAT 3 target genes by upregulating miR-26a expression. (56)

4.3.2. Serum

The serum level of miR-26a echoes the changes to their tissue level. Zhou *et al.*, compared a large cohort of patients' serum, and found that miR-26a is significantly downregulated in patients with HBV related HCC when compared to healthy control and patients with chronic HBV infection with or without cirrhosis. The serum miRNA-26a level is able to distinguish HBV-HCC from the other groups with an AUC of 0.665. (70)

4.4. miR-221

4.4.1. Tissue

Upregulation of miR-221 had previously been found in various tumor types such as glioblastoma, (71) bladder cancer, (72) papillary thyroid tumor (73) and pancreatic cancers. (74) Gramantieri *et al.*, used miRNA microarrays to compare a collection of cirrhosis-associated HCC tissues, to their corresponding non-malignant cirrhotic tissues. They found miR-221 to be upregulated in 83% of HCC samples when compared to their matched cirrhotic tissues. (75)

MiR-221 overexpression can promote malignant cell proliferation by inhibiting the cyclin-dependent kinase inhibitors, CDKN1B/p27, (76-79) CDKN1C/p57, (80) and protein c-kit. (81) It also inhibits apoptosis by down regulating the pro-apoptotic protein Bmf. (82) In HCC, downregulation of both CDKN1B/p27 and CDKN1C/p57 has been associated with more aggressive and advanced tumor stage, poorer prognosis and shorter disease-free survival. (83-85) This is likely to be a result of miR-221 interacting with their respective mRNAs, which leads to downregulation of CDKN1B/p27 and CDKN1C/p57 and hence promotes cells entering S-phase of the cell cycle and subsequent tumor growth. (80)

4.4.2. Serum

Li *et al.*, compared serum miR-221 expression between 46 HCC patients (30 are HBV-related HCC) and 20 healthy controls. In 76% of HCC patients' serum the miR-221 level was upregulated at least 2-fold (average 4.8 fold increase). When separated into high and low expression groups based on average fold change of 4.8, the authors found that a high level of miR-221 expression is significantly correlated with cirrhosis, a larger tumor size (≥5cm) and a more advanced tumor stage. Furthermore, high levels of miR-221 expression were associated with significantly lower overall survival and were an independent factor in predicting the overall survival in HCC patients. (86)

Luo *et al.*, compared the serum miR-221 levels in 85 HCC patients (75 are HBV-related HCC) with 85 healthy controls, and found that although miR-221 level was higher in the HCC group, the difference was not statistically significant. (87)

4.5. miR-224

4.5.1. Tissue

miR-224 is one of the better-understood miRNAs in HCC and many other cancers. It has been associated with a variety of carcinogenic processes including cell differentiation, transcription, apoptosis, growth and proliferation. (88)

Ladeiro *et al.*, (89) and Murakami *et al.*, (37) showed in their cohorts with similar numbers of HBV and HCV-related HCC patients that tissue miR-224 in the cancer is significantly upregulated compared to adjacent normal liver. (37, 89) The upregulation of miR-224 in HepG2 cell line promotes cell proliferation, invasion and migration. (90) When the HCC cell line THLE-3 was transfected with a miR-224 inhibitor, its API-5 expression was significantly increased and showed protection against UV-induced apoptosis. (91)

In contrast, in *in vivo* assays in the same study showed that by transfecting a colorectal cell line with low endogenous miR-224 expression, with miR-224 precursor, cells had significantly increased apoptotic rate as a result of miR-224's inhibition of the apoptosis inhibitor 5 (API-5) gene.

Interestingly, miR-224 could also significantly promote cell proliferation, in

combination with its ability of promoting apoptosis, the net result was a fairly constant number of viable cells compared to control but decreased cell viability. (91) The Myc oncogene is another regulator that has been demonstrated to have this seemingly contradictory role in a cell. (92) It is possible that miR-224, like Myc, can promote cell competition by eliciting surrounding cell apoptosis while promoting tumor cell proliferation and results in a net clonal expansion of the tumor.

4.5.2. Serum

Serum miR-224 have been shown to be elevated in HCC and cirrhotic patients compared to healthy control. (93, 94) However, its level was very low and Gui *et al.*, found that although patients with chronic cirrhosis had a significantly higher level compared to healthy control, there was no statistically significance between HCC patients and healthy control. (93)

4.6. miR-500

4.6.1. Tissue

Using miRNA microarray technology studying the global miRNA expression of different stages of neonatal mouse livers, Yamamoto *et al.*, found that miR-500 is a potential oncofetal miRNA. Its level is normally high in fetal liver and subsequently downregulated during developmental process, however, its expression can significantly increase with cirrhosis and HCC, but not in chronic hepatitis, when compared to adjacent normal tissues. (95)

4.6.2. Serum

Yamamoto *et al.*, further tested 10 patients' serum and found that 3 of the patients had a significantly higher level of miR-500 expression. All 3 patients' level returns back to normal after surgical resection. More studies need to be done on larger cohorts to establish the sensitivity, specificity and roles of miR-500 in HCC.

4.7. miR-885-5p

4.7.1. Tissue

Downregulation of tissue miR-885-5p levels have been shown to be significantly correlated with HCC recurrence (>50% of the patient samples are HCV positive, only 7.5% are HBV positive) within 3 years after liver transplantation. (96) However, we have limited understanding of miR-885-5p's role in hepatocarcinogenesis. One study discovered that miR-885-5p's downregulation is associated with primary neuroblastoma and that miR-885-5p can activate the cell cycle regulator p53, inhibit cyclin-dependent kinase 2 (CDK2) and the mini-chromosome maintenance protein 5 (MCM5). (97)

4.7.2. Serum

Gui *et al.* showed that the serum level of miR-885-5p is significantly upregulated in patients with chronic HBV infection, HBV-related HCC and liver cirrhosis compared to healthy adults. However, no significant differences were seen between the 3 groups of patients. Serum miR-885-85p yielded a diagnostic accuracy AUC of 0.904, with 90.53% sensitivity and 79.17% specificity, in distinguishing patients with HBV related liver pathologies from healthy adults. (93) This study has demonstrated that serum miR-885-5p level can help distinguishing healthy adults from patients with HBV related liver pathologies.

4.8. miR-15b, -130b

4.8.1. Tissue

In silico analysis of the HCC miRNA microarray database in National Centre for Biotechnology Information Gene Expression Omnibus, including information from both HBV and HCV infected HCC, found that miR-15b is significantly upregulated in HCC tissues. (98) Comparing a cohort of 96 pairs of HBV-related HCC tissues and their adjacent normal tissue, Liu *et al.*, found that miR-15b and miR-130b were significantly upregulated in HCC tissue in high copy number (>100 copies/10pg input RNA). This remained true for HCC with low AFP secretion (<400ng/ml). (94)

CD133+ HCC cells had been found to possess stem cell-like properties such as self-renewal and differentiation into non-hepatocyte-like, angiomyogenic like lineages. Furthermore, they have greater ability to

initiate tumors *in vivo* and shown to be more resistant to chemotherapy. (99-102) Ma *et al.*, demonstrated that miR-130b was significantly overexpressed in CD133+ HCC cell lines when compared to CD133- cells. CD133- HCC cell lines showed a greater potential for self-renewal, superior resistance to chemotherapy and enhanced tumorigenicity via the Akt/PKB and Bcl-2 pathways when they were transduced with miR-130b. (102) When antagonising miR-130b was transfected into CD133+ HCC cells, the opposite effects were observed. (102)

The potential roles of miR-15b in hepatocarcinogenesis are less understood. One study found that fibrotic liver tissues had significantly lower miR-15b level when compared to normal liver tissues. It demonstrated that miR-15b could target Bcl-2 and Caspase signalling pathways to induce apoptosis in rat hepatic stellate cells (HSCs). When miR-15b expression is downregulated, the increased Bcl-2 and Caspase signalling can promote HSCs proliferation and inordinate extracellular matrix production, which can lead to liver fibrosis. (103)

4.8.2. Serum

Liu *et al.* conducted a comprehensive study on serum miR-15b and miR-130b in a cohort of mainly HBV-related HCC patients. They discovered that serum miR-15b level was able to distinguish HCC from healthy patients with a sensitivity of 98.3%, specificity of 15.3% and AUC of 0.485. Serum level of miR-130b gave a sensitivity of 87.7%, specificity of 81.4% and an AUC of 0.913. However, when both were used together, they were able to distinguish HCC patients from healthy controls with a sensitivity of 98.3%, specificity of 91.5% and an AUC of 0.981. This diagnostic accuracy remains fairly constant when tested in groups of patients with a normal level of AFP (<20ng/ml) and patients with early stage HCC (TNM I or II). (94) This study had demonstrated the usefulness of serum miRNA in diagnosing HBV-related HCC compared to traditional AFP measurement and the potential of serum miRNA in clinical practice. (94)

4.9. miR-21

4.9.1. Tissue

Numerous studies have established that miR-21 expression is significantly upregulated in HCC compared to their adjacent normal tissue. (89, 94, 104) Liu *et al.*, studied the miR-21 expression in HBV related HCC patients and found their increased expression remained true in patients with low AFP levels (<400ng/ml). (94) Meng *et al.* used miRNA microarrays and found that HCC tissues (unspecified underlying aetiology) and its cell lines overexpressed miR-21. Increased miR-21 expression in tumor cell lines, by transfection with precursor miR-21, is associated with increased tumor cell invasion, migration and proliferation. (104) Connolly *et al.*, further discovered that miR-21 is over expressed in 100% of HBV associated HCC samples (n=19) and that knocking down miR-21 was associated with a 50% reduction in cell proliferation. (43) MiR-21 is likely to be involved in HCC tumorigenesis by its inhibitory effect on phosphatase and tensin homolog tumor suppressor (PTEN). It has been observed that PTEN level rises when miR-21 is inhibited and tumor cell progression is delayed. (104)

4.9.2. Serum

The serum level of miR-21 was significantly upregulated in HCC patients and decreased post HCC resection, although not statistically significant. (94, 105) Using miR-21 as a diagnostic marker for HCC yielded a specificity of 89.5%, sensitivity of 71.2% and an AUC of 0.865. (94)

4.10. miR-223

4.10.1. Tissue

MiR-223 was downregulated in HCC compared to normal liver tissues irrespective of underlying viral association. (106) It was found to be a likely suppressor of Stathmin1 (STMN1), which is a key microtubule-regulatory protein that controls cellular proliferation and cell cycle. STMN1 over-expression has been shown to correlate significantly with HCC vascular invasion, high tumor grades and early recurrences. (107) Wong *et al.* found that they could significantly reduce HCC cell viability by

knocking down STMN1 or by transfecting cells with miR-223 ($p<0.01$). (106)

4.10.2. Serum

Li *et al.*, (37) and Xu *et al.*, (41) studied the serum miR-223 expression in a large cohort of mainly HBV infected patients and found that it is significantly elevated in patients HBV-positive HCC when compared to healthy adults. (51, 105) When serum level of miR-223 was measured with miR-23b, -423, -375, -23a, and -342-3p, they were able to accurately distinguish patients HBV-positive HCC from healthy adults with an AUC of 0.999. (51)

4.11. miR-122

4.11.1. Tissue

MiR-122 is a liver specific miRNA and it is significantly downregulated in HCC tissues. (108, 109) All common HCC cell lines, which none were from patients with HCV infection, also showed downregulation of miR-122. (110) A significant reduction of miR-122 expression has been associated with advanced tumor with intrahepatic metastasis. Tsai *et al.*, demonstrated that miR-122 can suppress angiogenesis by regulating ADAM17, a disintegrin and metalloprotease involved in regulating cell adhesion, migration and metastasis. (111) MiR-122 can also regulate ADAM10, Serum Response Factor (SRF), Insulin-like growth factor 1 receptor (IGF1R), and cyclin G1; their deregulation has been linked to tumorigenesis. (112, 113) Bai *et al.*, demonstrated that increasing expression of miR-122 in HCC cell lines could sensitise cells to be more susceptible to Sorafenib. (112) Clinically, the level of miR-122 expression was highly heterogeneous in HCC samples. HCC patients with low tissue level of miR-122 had a significantly lower overall survival, (114) and Coulouarn *et al.*, showed that in patients with HBV-related HCC, suppression of tissue miR-122 could be correlated with increased tumor size, grade and metastatic properties. (114) Varnholt *et al.*, looked at the HCC tissues from patients with underlying HCV infection and found that miR-122 level was significantly increased rather than decreased compared to healthy controls.

4.11.2. Serum

Luo *et al.*, compared serum from 85 HCC patients (75 are HBV-related HCC) with 85 matched healthy adults and found that the serum miR-122 level was significantly downregulated in HCC patients, however, it alone was not superior to AFP for HCC diagnosis. (87) In patients with chronic HCV hepatitis and non-alcoholic fatty liver disease, serum miR-122 was significantly upregulated. (115, 116) It is hence possible that miR-122 expression is aetiology dependent; being downregulated in HBV-related HCC and upregulated in HCV related HCC.

In contrast, Xu *et al.*, found that serum miR-122 was significantly upregulated in a cohort of mainly HBV-related HCC patients. (105) Along with serum miR-21 and miR-223, which were also upregulated, the 3 miRNAs showed a greater increase in patients with chronic hepatitis B and chronic cirrhosis, to a greater degree than in patients with HCC. The author postulated that serum miR-122 and -223 level reflected hepatocyte injury rather than malignancy and because miRNAs were very stable in the circulation, their levels could accumulate with time. (105) This notion was supported by two other papers, which found miR-122 among several other miRNA levels to be upregulated with liver injury. (31, 117)

4.12. miR-16

4.12.1. Tissue

MiR-16 has been found to reduce the expression levels of cyclin D1 (CD1) and lead to cell cycle arrest and subsequent apoptosis. (118) The miR-16 expression is downregulated in hepatic satellite cells, an important mediator causing liver fibrosis. (119) Qu *et al.*, further discovered that both HCC (mixed aetiology, 66 HCV, 20 HBV, 19 others) and chronic liver disease patients have significantly lower levels of serum miR-16 compared to healthy individuals. The level in HCC patients is further significantly lower than that in patients with chronic liver disease.

4.12.2. Serum

When serum miR-16 levels were used as a diagnostic tool for HCC, it had a sensitivity

of 79.1% (HCC <3mm), 66.1% (HCC >3mm) and a specificity of 88.8%. However, when combined with AFP, AFP-L3, and DCP levels, its sensitivity increased to 88.4% (<3mm), 93.5% (>3mm) at a small expense of specificity to 78.5%. (120) Gui *et al.*, found conflicting results in a group of predominantly HBV-related HCC patients. They found that the serum miR-16 level was actually significantly elevated rather than suppressed. One possible explanation was the different patient demographics, one population had predominantly HCV-related HCC, whilst the other population had predominant HBV-related HCC (Qu *et al.*, Gui *et al.* respectively). (93)

4.13. miR-195, -199a

4.13.1. Tissue

The expression of miR-195 and miR-199a were found to be significantly downregulated in HCC tissues. (37, 75, 121) Like miR-16, both miR-195 and miR-199a are cell cycle suppressors that can suppress HCC tumor growth *in vivo*. Xu *et al.*, found that miR-195 may block the G1/S transition by repressing tumor suppressor retinoblastoma/E2F signalling through targeting CD1, cyclin-dependent kinase 6 (CDK6), and transcription factor E2F3 (121). Fornari *et al.*, found that miR-199a could lead to G1 phase cell cycle arrest by targeting mammalian target of rapamycin (mTOR) and c-MET. (122)

4.13.2. Serum

Like miR-16, serum levels of miR-195 and miR-199a were found to be significantly lower in HCC patients when compared to healthy individuals and patients with chronic liver disease. Although serum miR-199a alone had limited HCC diagnostic sensitivity (sensitivity of less than 57%), when coupled with the miR-16, AFP, AFP-L3 and DCP combination described above, sensitivity increased further to 93% for small HCC (<3mm), and 95.2% for HCC >3mm and a specificity of 72.9%. (120)

4.14. miR--27a, -192, -801

4.14.1. Tissue

MiR-192 is overexpressed in HepG2 cell line, which is a HCC cell line generated from a HBV patient. MiR-192 can repress gene ERCC3 and ERCC4 expression, both of which play a key role in the DNA nucleotide excision repair system. Impairment of this system can lead to carcinogenesis. (123) However, more research is needed to compare miR-192 expression between HCC and healthy tissues. We also know very little about miR-27a and miR-801 in tissue.

4.14.2. Serum

Zhou *et al.*, used microarray and screened blood samples from a large cohort of HBV-related HCC patients versus healthy controls, chronic hepatitis B and cirrhosis patients. They identified a panel of microRNAs (miR-21, -26a, -27a, 122, -192, -223, -801) that provided a high diagnostic accuracy of HCC irrespective of disease stage. Even at a very early stage (i.e. Barcelona Liver Cancer Stage of 0), the panel provides a diagnostic accuracy of 0.888. The panel can also distinguish HCC from healthy with an AUC of 0.941, chronic hepatitis B (AUC=0.842) and cirrhosis (AUC=0.884). (70).

4.15. miR-25, -375, let-7f

4.15.1. Tissue

MiR-25 belongs to a cluster of 3 miRNAs consisting of miR-25, -93 and -106b. These are located on chromosome 7 within the minichromosome maintenance protein 7 (MCM7) gene. (42) The MCM7 gene and the miR-106b-25 cluster are overexpressed in HCC cell lines and HBV-related HCC tissue. (104, 106, 124) This cluster of miRNAs has been shown to be involved in regulating cell proliferation in prostate, gastric cancer and HCC. (42, 125, 126) Li *et al.*, demonstrated that although inhibiting each individual members of the miR-106b-25 cluster will result in suppressed cell proliferation, when all 3 members were inhibited together, a greater degree of suppression can be seen. They also demonstrated that miR-25 can inhibit Bim, which is a Bcl-2 family protein that promotes apoptosis, concluding that upregulated miR-25 expression in HCC hinders cell apoptosis and promotes cancer progression. (42, 127)

MiR-375 can regulate the Yes-Associated Protein (YAP) oncogene expression. Its expression is significantly downregulated in HCC compared to adjacent non-tumor tissue, and as a result YAP production is upregulated and in turn potentiates hepatocarcinogenesis. (128, 129) YAP is a transcriptional co-activator that can promote HCC cell proliferation and survival and is overexpressed in approximately 62% of HCC patients. Furthermore, miR-375 can suppress the Astrocyte Elevated Gene-1 (AEG-1) oncogene, which has been found to be associated with >90% of HCC, among many other cancers, and is associated with poor clinical outcome. (130) Our knowledge on AEG-1 is limited, however, it has been found to be able to activate the Wnt/β-catenin pathway, which is important for HCC progression. (131)

The let-7 family/cluster (let-7a-1, -2, -3, let-7b, let-7c, let-7d, let-7e, let-7f-1, -2, let-7g, let-7i and miR98) of miRNAs is one of the earliest discovered in *C. elegans* and, subsequently humans. (132, 133) Mature let-7 is highly conserved across animal species and is involved with various different functions across species, notably the stem-cell differentiation in *C. elegans* and as tumor suppressors in several tumors. (134-136) The let-7 down-regulation has been correlated to several human cancers (137-142) and In HCC, the let-7 family miRNAs are significantly downregulated when compared to adjacent non-tumor or cirrhotic tissues. (37, 75) One of several ways let-7 suppression may lead to carcinogenesis is through its regulation of the RAS/MAP kinase pathway. The RAS/MAP kinase pathway is involved in cell proliferation, differentiation, apoptosis, and tissue development. RAS is found to be mutated in approximately 30% of all human cancers; its mutation can lead to uncontrolled cell growth. (143, 144) Let-7 family miRNAs are regulators of RAS; therefore downregulated let-7 may result in increased tumorigenesis. (137)

Shimizu *et al.*, discovered that let-7c and let-7g can regulate Bcl-xL expression by targeting the 3'UTR region of Bcl-xL mRNA. Bcl-xL is an anti-apoptotic member of the Bcl family and its over-expression in HCC is likely due to decreased let-7c and let-7g expression. (141) Shimizu *et al.*, have further demonstrated that they could potentiate Sorafenib induced apoptosis by transfecting Huh7 cells with let-7c. (141) Reduced levels of let-7f were previously found to be associated with highly invasive and metastatic gastric cancers. When let-7f expression is increased through lentivirus transfection *in vivo*, gastric cancer metastasis can be inhibited. (145) However, to our knowledge, no one has investigated the role of let-7f in HCC yet.

4.15.2. Serum

Li *et al.*, used next generation sequencing (Illumina Solexa), followed by qRT-PCR on blood samples collected from a large group of cohort consisting of healthy control, HBV-related HCC, HBV- and HCV- infected patients. They discovered that serum levels of miR-25, -375 and let-7f were all significantly upregulated compared to healthy control and serum miR-375 alone could distinguish HBV-related HCC patients from healthy controls with an AUC of 0.96 (specificity of 96%; sensitivity of 100%). When serum miR-25 and let-7f were tested together with miR-375, it increased the AUC to 0.9967+/- 0.015 (specificity 99.1%; sensitivity 99.1%). They could also distinguish HBV-infected patients from healthy control with equally high accuracy (AUC 0.9967 +/- 0.015, specificity 99.1%, sensitivity 97.9%). This is the highest diagnostic accuracy we have seen in literature for HCC diagnosis so far. (51)

5. DISCUSSION

The field of circulating miRNA is relatively new but it has been developing rapidly. Taken together, the data suggest many circulating miRNAs are promising biomarkers that are superior to AFP and can allow an earlier diagnosis and monitoring of HCC in a non-invasive manner. Many circulating miRNAs have been shown to be able to distinguish HCC patients from healthy individuals and patients with chronic cirrhosis with extremely high accuracy especially in patients with

HBV infection. Undoubtedly, the future of HCC management needs to focus on earlier diagnosis, better indicators of prognosis and improved treatments.

This review has highlighted that in order to identify and compare aberrantly expressed circulating miRNAs, a large patient sample with an age, gender and race matched control group, along with knowledge of hepatitis serology and underlying liver disease is essential. (146) As HCC is a heterogeneous disease with various aetiologies, a small sample size could easily obscure true associations and might explain the inconsistencies in the various reports. Furthermore, HBV and HCV-related HCC often express a different miRNA profile. The majority of the studies were conducted on HBV-related mainland Chinese patients. Although it reflects the demographics of the disease, patients with other aetiology and ethnicities need to be recruited in the future to generate a more coherent picture of the role of miRNA in hepatocarcinogenesis. It is likely that different panels of miRNAs would need to be employed in the future for patients with different ethnic backgrounds and HCC aetiologies.

Another obstacle for comparing miRNA expression across different studies is the lack of a standardised internal reference. Many studies used U6 RNA, which is normally highly expressed. However, RNA is unstable in the plasma, and may be subject to degradation if the sample handling is not appropriate and timely. (147) Other studies have used stable and highly expressed miRNAs as their internal control, such as miR-16, -181a, -181c, -638 and -1228. These need to be validated to reach a consensus as to which one is more appropriate and should be used by all researchers in the future.

Future studies will also need to correlate the circulating miRNA level to other aspects of HCC management beyond diagnosis and monitoring such as prognosis, likelihood of recurrence and response to chemotherapy. Ji *et al.*, discovered patients with low tissue miR-26 expressing tumors respond better to interferon therapy. (55) This supports the rationale that miRNAs may have a potential to distinguish a subpopulation of patients who may respond favourably to a systemic therapy. The ability to predict tumor recurrence and to prognosticate a patient's disease also has other implications such as decision for liver transplantation or other forms of treatments. The result will be a more targeted treatment, which can improve patient care, and better distribution of resources.

Finally, the areas that future serum miRNA studies need to focus on are 1) recruiting larger cohorts of patients with different ethnicities, 2) astutely record patients' HCC aetiology and the need to include it into the analysis, 3) correlate serum miRNA level to other clinical parameters, and lastly, 4) the use and comparison of standardised internal references.

6. ACKNOWLEDGEMENT

We would like to thank Dr Jodie Palmer for assistance. Dr Tsao is funded by an Australian Postgraduate Award & Foundation for Surgery Research Fellowship (2014). Dr Behren is funded by a Cure Cancer Foundation Australia Fellowship.

7. REFERENCES

1. A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward and D. Forman: Global cancer statistics. CA Cancer J Clin, 61(2), 69-90 (2011)
DOI: 10.3322/caac.20107
2. J. F. Perz, G. L. Armstrong, L. A. Farrington, Y. J. Hutin and B. P. Bell: The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol, 45(4), 529-38 (2006)
DOI: 10.1016/j.jhep.2006.05.013
3. G. Manghisi, Elba, S., Mossa, A., Giorgio, A., Aloisio, V., Perrotta, A., Tardio, B., Del Naja, C., Ca- turelli, E., Calandra, M., Castellano, L., De Sio, I., Capuano, G., Pomponi, D.,

Casti- glione, F., Cocchia, P., Farinati, F., Rinaldi, M., Adinolfi, L.E., Ragone, E., Felder, M., Zancanella, L., Pasquale, G., Stanzione, M., Marone, G., DAngelo, V., Gaeta, G.B., Giolitto, G., Lamborgese, B., Manzione, L., Russo, M., Colurcio, R., Veltri, E., Izzo, F: A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology, 28(3), 751-5 (1998)
DOI: 10.1002/hep.510280322

4. A. Budhu, 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 and X. W. Wang: Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology, 47(3), 897-907 (2008)
DOI: 10.1002/hep.22160

5. S. T. Fan, C. Mau Lo, R. T. Poon, C. Yeung, C. Leung Liu, W. K. Yuen, C. Ming Lam, K. K. Ng and S. Ching Chan: Continuous improvement of survival outcomes of resection of hepatocellular carcinoma: a 20-year experience. Ann Surg, 253(4), 745-58 (2011)
DOI: 10.1097/SLA.0b013e3182111195

6. J. Bruix, M. Sherman and A. A. f. t. S. o. L. D. Practice Guidelines Committee: Management of hepatocellular carcinoma. Hepatology, 42(5), 1208-36 (2005)
DOI: 10.1002/hep.20933

7. D. Cherqui, A. Laurent, N. Mocellin, C. Tayar, A. Luciani, J. T. Van Nhieu, T. Decaens, M. Hurtova, R. Memeo, A. Mallat and C. Duvoux: Liver resection for transplantable hepatocellular carcinoma: long-term survival and role of secondary liver transplantation. Ann Surg, 250(5), 738-46 (2009)
DOI: 10.1097/SLA.0b013e3181bd582b

8. L. Y. Yang, F. Fang, D. P. Ou, W. Wu, Z. J. Zeng and F. Wu: Solitary large hepatocellular carcinoma: a specific subtype of hepatocellular carcinoma with good outcome after hepatic resection. Ann Surg, 249(1), 118-23 (2009)
DOI: 10.1097/SLA.0b013e3181904988

9. R. S. Soong, M. C. Yu, K. M. Chan, H. S. Chou, T. J. Wu, C. F. Lee, T. H. Wu and W. C. Lee: Analysis of the recurrence risk factors for the patients with hepatocellular carcinoma meeting University of California San Francisco criteria after curative hepatectomy. World J Surg Oncol, 9, 9 (2011)
DOI: 10.1186/1477-7819-9-9

10. A. B. Benson, 3rd, T. A. Abrams, E. Ben-Josef, P. M. Bloomston, J. F. Botha, B. M. Clary, A. Covey, S. A. Curley, M. I. D'Angelica, R. Davila, W. D. Ensminger, J. F. Gibbs, D. Laheru, M. P. Malafa, J. Marrero, S. G. Meranze, S. J. Mulvihill, J. O. Park, J. A. Posey, J. Sachdev, R. Salem, E. R. Sigurdson, C. Sofocleous, J. N. Vauthey, A. P. Venook, L. W. Goff, Y. Yen and A. X. Zhu: NCCN clinical practice guidelines in oncology: hepatobiliary cancers. J Natl Compr Canc Netw, 7(4), 350-91 (2009)

11. J. A. Marrero: Screening tests for hepatocellular carcinoma. Clin Liver Dis, 9(2), 235-51, vi (2005)

12. R. K. Sterling, E. C. Wright, T. R. Morgan, L. B. Seeff, J. C. Hoefs, A. M. Di Bisceglie, J. L. Dienstag and A. S. Lok: Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. Am J Gastroenterol, 107(1), 64-74 (2012)
DOI: 10.1038/ajg.2011.312

13. X. Liu, Y. Cheng, W. Sheng, H. Lu, Y. Xu, Z. Long, H. Zhu and Y. Wang: Clinicopathologic features and prognostic factors in alpha-fetoprotein-producing gastric cancers: analysis of 104 cases. J Surg Oncol, 102(3), 249-55 (2010)
DOI: 10.1002/jso.21624

14. M. El-Bahrawy: Alpha-fetoprotein-producing non-germ cell tumours of the female genital tract. Eur J Cancer, 46(8), 1317-22 (2010)
DOI: 10.1016/j.ejca.2010.01.028

15. J. Bruix, M. Sherman and D. American Association for the Study of Liver:

Management of hepatocellular carcinoma: an update. Hepatology, 53(3), 1020-2 (2011)
DOI: 10.1002/hep.24199

16. T. Behne and M. S. Copur: Biomarkers for hepatocellular carcinoma. Int J Hepatol, 2012, 859076 (2012)
DOI: 10.1155/2012/859076

17. G. Shiota and N. Miura: Biomarkers for hepatocellular carcinoma, Clinical Journal of Gastroenterology, 5(3), 177-182 (2012)

18. N. Iizuka, M. Oka, H. Yamada-Okabe, N. Mori, T. Tamesa, T. Okada, N. Takemoto, A. Tangoku, K. Hamada, H. Nakayama, T. Miyamoto, S. Uchimura and Y. Hamamoto: Comparison of gene expression profiles between hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma by oligonucleotide microarray data on the basis of a supervised learning method. Cancer Res, 62(14), 3939-44 (2002)

19. M. Bellodi-Privato, M. S. Kubrusly, J. T. Stefano, I. C. Soares, A. Wakamatsu, A. C. Oliveira, V. A. Alves, T. Bacchella, M. C. Machado and L. A. D'Albuquerque: Differential gene expression profiles of hepatocellular carcinomas associated or not with viral infection. Braz J Med Biol Res, 42(12), 119-1127 (2009)

20. X. Chen, S. T. Cheung, S. So, S. T. Fan, C. Barry, J. Higgins, K. M. Lai, J. Ji, S. Dudoit, I. O. Ng, M. Van De Rijn, D. Botstein and P. O. Brown: Gene expression patterns in human liver cancers. Mol Biol Cell, 13(6), 1929-39 (2002)
DOI: 10.1091/mbc.02-02-0023.

21. D. P. Bartel: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 116(2), 281-97 (2004)
DOI: 10.1016/S0092-8674(04)00045-5

22. R. C. Lee, R. L. Feinbaum and V. Ambros: The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell, 75(5), 843-54 (1993)
DOI: 10.1016/0092-8674(93)90529-Y

23. W. Wu, M. Sun, G. M. Zou and J. Chen: MicroRNA and cancer: Current status and prospective. Int J Cancer, 120(5), 953-60 (2007)
DOI: 10.1002/ijc.22454

24. J. Yu, F. Wang, G. H. Yang, F. L. Wang, Y. N. Ma, Z. W. Du and J. W. Zhang: Human microRNA clusters: genomic organization and expression profile in leukemia cell lines. Biochem Biophys Res Commun, 349(1), 59-68 (2006)
DOI: 10.1016/j.bbrc.2006.07.207

25. R. N. Aravalli, C. J. Steer and E. N. Cressman: Molecular mechanisms of hepatocellular carcinoma. Hepatology, 48(6), 2047-63 (2008)
DOI: 10.1002/hep.22580

26. M. Negrini, M. Ferracin, S. Sabbioni and C. M. Croce: MicroRNAs in human cancer: from research to therapy. J Cell Sci, 120(Pt 11), 1833-40 (2007)
DOI: 10.1242/jcs.03450

27. L. He, J. M. Thomson, M. T. Hemann, E. Hernando-Monge, D. Mu, S. Goodson, S. Powers, C. Cordon-Cardo, S. W. Lowe, G. J. Hannon and S. M. Hammond: A microRNA polycistron as a potential human oncogene. Nature, 435(7043), 828-33 (2005)
DOI: 10.1038/nature03552

28. L. Gailhouste, L. Gomez-Santos and T. Ochiya: Potential applications of miRNAs as diagnostic and prognostic markers in liver cancer. Front Biosci (Landmark Ed), 18, 199-223 (2013)
DOI: 10.2741/4096

29. F. Borel, P. Konstantinova and P. L. Jansen: Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. J Hepatol, 56(6), 1371-83 (2012)
DOI: 10.1016/j.jhep.2011.11.026

30. N. Kosaka, H. Iguchi and T. Ochiya: Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci, 101(10), 2087-92 (2010)
DOI: 10.1111/j.1349-7006.2010.01650.x

31. K. Wang, S. Zhang, B. Marzolf, P. Troisch, A. Brightman, Z. Hu, L. E. Hood and D. J. Galas: Circulating microRNAs, potential biomarkers for drug-induced liver injury. Proc Natl Acad Sci U S A, 106(11), 4402-7 (2009)
DOI: 10.1073/pnas.0813371106

32. Y. Zhang, Y. Jia, R. Zheng, Y. Guo, Y. Wang, H. Guo, M. Fei and S. Sun: Plasma microRNA-122 as a biomarker for viral-, alcohol-, and chemical-related hepatic diseases. Clin Chem, 56(12), 1830-8 (2010)
DOI: 10.1373/clinchem.2010.147850

33. P. S. Mitchell, 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 and M. Tewari: Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A, 105(30), 10513-8 (2008)
DOI: 10.1073/pnas.0804549105

34. X. Chen, 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 and C. Y. Zhang: Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res, 18(10), 997-1006 (2008)
DOI: 10.1038/cr.2008.282

35. C. Chen, D. A. Ridzon, A. J. Broomer, Z. Zhou, D. H. Lee, J. T. Nguyen, M. Barbisin, N. L. Xu, V. R. Mahuvakar, M. R. Andersen, K. Q. Lao, K. J. Livak and K. J. Guegler: Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res, 33(20), e179 (2005)
DOI: 10.1093/nar/gni178

36. L. Gailhouste and T. Ochiya: Cancer-related microRNAs and their role as tumor suppressors and oncogenes in hepatocellular carcinoma. Histol Histopathol, 28(4), 437-51 (2013)

37. Y. Murakami, T. Yasuda, K. Saigo, T. Urashima, H. Toyoda, T. Okanoue and K. Shimotohno: Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene, 25(17), 2537-45 (2006)
DOI: 10.1038/sj.onc.1209283

38. Y. Hayashita, H. Osada, Y. Tatematsu, H. Yamada, K. Yanagisawa, S. Tomida, Y. Yatabe, K. Kawahara, Y. Sekido and T. Takahashi: A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res, 65(21), 9628-32 (2005)
DOI: 10.1158/0008-5472.CAN-05-2352

39. E. Bandres, E. Cubedo, X. Agirre, R. Malumbres, R. Zarate, N. Ramirez, A. Abajo, A. Navarro, I. Moreno, M. Monzo and J. Garcia-Foncillas: Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Mol Cancer, 5, 29 (2006)
DOI: 10.1186/1476-4598-5-29

40. S. Volinia, 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 and C. M. Croce: A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A, 103(7), 2257-61 (2006)
DOI: 10.1073/pnas.0510565103

41. C. W. Wu, Y. J. Dong, Q. Y. Liang, X. Q. He, S. S. Ng, F. K. Chan, J. J. Sung and J. Yu: MicroRNA-18a attenuates DNA damage repair through suppressing the expression of ataxia telangiectasia mutated in colorectal cancer. PLoS One, 8(2), e57036 (2013)
DOI: 10.1371/journal.pone.0057036

42. Y. Li, W. Tan, T. W. Neo, M. O. Aung, S. Wasser, S. G. Lim and T. M. Tan: Role

of the miR-106b-25 microRNA cluster in hepatocellular carcinoma. Cancer Sci, 100(7), 1234-42 (2009)
DOI: 10.1111/j.1349-7006.2009.01164.x

43. E. Connolly, M. Melegari, P. Landgraf, T. Tchaikovskaya, B. C. Tennant, B. L. Slagle, L. E. Rogler, M. Zavolan, T. Tuschl and 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(3), 856-64 (2008)
DOI: 10.2353/ajpath.2008.080096

44. H. Su, J. R. Yang, T. Xu, J. Huang, L. Xu, Y. Yuan and S. M. Zhuang: MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. Cancer Res, 69(3), 1135-42 (2009)
DOI: 10.1158/0008-5472.CAN-08-2886

45. W. H. Liu, S. H. Yeh, C. C. Lu, S. L. Yu, H. Y. Chen, C. Y. Lin, D. S. Chen and P. J. Chen: MicroRNA-18a prevents estrogen receptor-alpha expression, promoting proliferation of hepatocellular carcinoma cells. Gastroenterology, 136(2), 683-93 (2009)
DOI: 10.1053/j.gastro.2008.10.029

46. W. Wu, M. Takanashi, N. Borjigin, S. I. Ohno, K. Fujita, S. Hoshino, Y. Osaka, A. Tsuchida and M. Kuroda: MicroRNA-18a modulates STAT3 activity through negative regulation of PIAS3 during gastric adenocarcinogenesis. Br J Cancer, 108(3), 653-61 (2013)
DOI: 10.1038/bjc.2012.587

47. A. Francavilla, L. Polimeno, M. Barone, A. Azzarone and T. E. Starzl: Hepatic regeneration and growth factors. J Surg Oncol Suppl, 3, 1-7 (1993)
DOI: 10.1002/jso.2930530503

48. J. D. Yager, Jr.: Oral contraceptive steroids as promoters or complete carcinogens for liver in female Sprague-Dawley rats. Environ Health Perspect, 50, 109-12 (1983)
DOI: 10.1289/ehp.8350109

49. L. Chen, J. Zheng, Y. Zhang, L. Yang, J. Wang, J. Ni, D. Cui, C. Yu and Z. Cai: Tumor-specific expression of microRNA-26a suppresses human hepatocellular carcinoma growth via cyclin-dependent and -independent pathways. Mol Ther, 19(8), 1521-8 (2011)
DOI: 10.1038/mt.2011.64

50. L. Li, Z. Guo, J. Wang, Y. Mao and Q. Gao: Serum miR-18a: a potential marker for hepatitis B virus-related hepatocellular carcinoma screening. Dig Dis Sci, 57(11), 2910-6 (2012)
DOI: 10.1007/s10620-012-2317-y

51. L. M. Li, Z. B. Hu, Z. X. Zhou, X. Chen, F. Y. Liu, J. F. Zhang, H. B. Shen, C. Y. Zhang and K. Zen: Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. Cancer Res, 70(23), 9798-807 (2010)
DOI: 10.1158/0008-5472.CAN-10-1001

52. M. Shigoka, 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 and M. Kuroda: Deregulation of miR-92a expression is implicated in hepatocellular carcinoma development. Pathol Int, 60(5), 351-7 (2010)
DOI: 10.1111/j.1440-1827.2010.02526.x

53. J. Kota, 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 and J. T. Mendell: Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell, 137(6), 1005-17 (2009)
DOI: 10.1016/j.cell.2009.04.021

54. J. Ji, 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 and X. W. Wang: MicroRNA expression, survival, and response to

interferon in liver cancer. N Engl J Med, 361(15), 1437-47 (2009)
DOI: 10.1056/NEJMoa0901282

55. X. Yang, L. Liang, X. F. Zhang, H. L. Jia, Y. Qin, X. C. Zhu, X. M. Gao, P. Qiao, Y. Zheng, Y. Y. Sheng, J. W. Wei, H. J. Zhou, N. Ren, Q. H. Ye, Q. Z. Dong and L. X. Qin: MicroRNA-26a suppresses tumor growth and metastasis of human hepatocellular carcinoma by targeting interleukin-6-Stat3 pathway. Hepatology, 58(1), 158-70 (2013)
DOI: 10.1002/hep.26305

56. H. B. El-Serag and K. L. Rudolph: Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology, 132(7), 2557-76 (2007)
DOI: 10.1053/j.gastro.2007.04.061

57. I. O. Ng, M. M. Ng, E. C. Lai and S. T. Fan: Better survival in female patients with hepatocellular carcinoma. Possible causes from a pathologic approach. Cancer, 75(1), 18-22 (1995)
DOI: 10.1002/1097-0142(19950101)75:1<18::AID-CNCR2820750105>3.0.CO;2-#

58. K. Dohmen, H. Shigematsu, K. Irie and H. Ishibashi: Longer survival in female than male with hepatocellular carcinoma. J Gastroenterol Hepatol, 18(3), 267-72 (2003)
DOI: 10.1046/j.1440-1746.2003.02936.x

59. P. Tangkijvanich, V. Mahachai, P. Suwangool and Y. Poovorawan: Gender difference in clinicopathologic features and survival of patients with hepatocellular carcinoma. World J Gastroenterol, 10(11), 1547-50 (2004)

60. C. Lu and R. S. Kerbel: Interleukin-6 undergoes transition from paracrine growth inhibitor to autocrine stimulator during human melanoma progression. J Cell Biol, 120(5), 1281-8 (1993)
DOI: 10.1083/jcb.120.5.1281

61. A. Rabinovich, L. Medina, B. Piura, S. Segal and M. Huleihel: Regulation of ovarian carcinoma SKOV-3 cell proliferation and secretion of MMPs by autocrine IL-6. Anticancer Res, 27(1A), 267-72 (2007)

62. K. Malinowska, H. Neuwirt, I. T. Cavarretta, J. Bektic, H. Steiner, H. Dietrich, P. L. Moser, D. Fuchs, A. Hobisch and Z. Culig: Interleukin-6 stimulation of growth of prostate cancer in vitro and in vivo through activation of the androgen receptor. Endocr Relat Cancer, 16(1), 155-69 (2009)
DOI: 10.1677/ERC-08-0174

63. F. R. Santer, K. Malinowska, Z. Culig and I. T. Cavarretta: Interleukin-6 trans-signalling differentially regulates proliferation, migration, adhesion and maspin expression in human prostate cancer cells. Endocr Relat Cancer, 17(1), 241-53 (2010)
DOI: 10.1677/ERC-09-0200

64. W. E. Naugler, T. Sakurai, S. Kim, S. Maeda, K. Kim, A. M. Elsharkawy and M. Karin: Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science, 317(5834), 121-4 (2007)
DOI: 10.1126/science.1140485

65. S. Abiru, K. Migita, Y. Maeda, M. Daikoku, M. Ito, K. Ohata, S. Nagaoka, T. Matsumoto, Y. Takii, K. Kusumoto, M. Nakamura, A. Komori, K. Yano, H. Yatsuhashi, K. Eguchi and H. Ishibashi: Serum cytokine and soluble cytokine receptor levels in patients with non-alcoholic steatohepatitis. Liver Int, 26(1), 39-45 (2006)
DOI: 10.1111/j.1478-3231.2005.01191.x

66. M. Soresi, L. Giannitrapani, F. D'Antona, A. M. Florena, E. La Spada, A. Terranova, M. Cervello, N. D'Alessandro and G. Montalto: Interleukin-6 and its soluble receptor in patients with liver cirrhosis and hepatocellular carcinoma. World J Gastroenterol, 12(16), 2563-8 (2006)

67. W. Lou, Z. Ni, K. Dyer, D. J. Tweardy and A. C. Gao: Interleukin-6 induces prostate cancer cell growth accompanied

by activation of stat3 signaling pathway. Prostate, 42(3), 239-42 (2000)
DOI:10.1002/(SICI)1097-0045(20000215)42:3<239::AID-PROS10>3.0.CO;2-G

68. J. Bromberg and T. C. Wang: Inflammation and cancer: IL-6 and STAT3 complete the link. Cancer Cell, 15(2), 79-80 (2009)
DOI: 10.1016/j.ccr.2009.01.009

69. J. Zhou, L. Yu, X. Gao, J. Hu, J. Wang, Z. Dai, J. F. Wang, Z. Zhang, S. Lu, X. Huang, Z. Wang, S. Qiu, X. Wang, G. Yang, H. Sun, Z. Tang, Y. Wu, H. Zhu and J. Fan: Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. J Clin Oncol, 29(36), 4781-8 (2011)
DOI: 10.1200/JCO.2011.38.2697

70. S. A. Ciafre, S. Galardi, A. Mangiola, M. Ferracin, C. G. Liu, G. Sabatino, M. Negrini, G. Maira, C. M. Croce and M. G. Farace: Extensive modulation of a set of microRNAs in primary glioblastoma. Biochem Biophys Res Commun, 334(4), 1351-8 (2005)
DOI: 10.1016/j.bbrc.2005.07.030

71. F. Gottardo, C. G. Liu, M. Ferracin, G. A. Calin, M. Fassan, P. Bassi, C. Sevignani, D. Byrne, M. Negrini, F. Pagano, L. G. Gomella, C. M. Croce and R. Baffa: Micro-RNA profiling in kidney and bladder cancers. Urol Oncol, 25(5), 387-92 (2007)
DOI: 10.1016/j.urolonc.2007.01.019

72. H. He, K. Jazdzewski, W. Li, S. Liyanarachchi, R. Nagy, S. Volinia, G. A. Calin, C. G. Liu, K. Franssila, S. Suster, R. T. Kloos, C. M. Croce and A. de la Chapelle: The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci U S A, 102(52), 19075-80 (2005)
DOI: 10.1073/pnas.0509603102

73. E. J. Lee, Y. Gusev, J. Jiang, G. J. Nuovo, M. R. Lerner, W. L. Frankel, D. L. Morgan, R. G. Postier, D. J. Brackett and T. D. Schmittgen: Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer, 120(5), 1046-54 (2007)
DOI: 10.1002/ijc.22394

74. L. Gramantieri, 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 and M. Negrini: Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. Cancer Res, 67(13), 6092-9 (2007)
DOI: 10.1158/0008-5472.CAN-06-4607

75. S. Galardi, N. Mercatelli, E. Giorda, S. Massalini, G. V. Frajese, S. A. Ciafre and M. G. Farace: miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. J Biol Chem, 282(32), 23716-24 (2007)
DOI: 10.1074/jbc.M701805200

76. J. K. Gillies and I. A. Lorimer: Regulation of p27Kip1 by miRNA 221/222 in glioblastoma. Cell Cycle, 6(16), 2005-9 (2007)
DOI: 10.4161/cc.6.16.4526

77. C. le Sage, R. Nagel, D. A. Egan, M. Schrier, E. Mesman, A. Mangiola, C. Anile, G. Maira, N. Mercatelli, S. A. Ciafre, M. G. Farace and R. Agami: Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. EMBO J, 26(15), 3699-708 (2007)
DOI: 10.1038/sj.emboj.7601790

78. Visone, L. Russo, P. Pallante, I. De Martino, A. Ferraro, V. Leone, E. Borbone, F. Petrocca, H. Alder, C. M. Croce and A. Fusco: MicroRNAs (miR)-221 and miR-222, both overexpressed in human thyroid papillary carcinomas, regulate p27Kip1 protein levels and cell cycle. Endocr Relat Cancer, 14(3), 791-8 (2007)
DOI: 10.1677/ERC-07-0129

79. F. Fornari, L. Gramantieri, M. Ferracin, A. Veronese, S. Sabbioni, G. A. Calin, G. L. Grazi, C. Giovannini, C. M. Croce, L. Bolondi and M. Negrini: MiR-221 controls CDKN1C/p57 and CDKN1B/p27 expression in human hepatocellular carcinoma. Oncogene, 27(43), 5651-61 (2008)
DOI: 10.1038/onc.2008.178

80. N. Felli, L. Fontana, E. Pelosi, R. Botta, D. Bonci, F. Facchiano, F. Liuzzi, V. Lulli, O. Morsilli, S. Santoro, M. Valtieri, G. A. Calin, C. G. Liu, A. Sorrentino, C. M. Croce and C. Peschle: MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. Proc Natl Acad Sci U S A, 102(50), 18081-6 (2005) DOI: 10.1073/pnas.0506216102

81. L. Gramantieri, F. Fornari, M. Ferracin, A. Veronese, S. Sabbioni, G. A. Calin, G. L. Grazi, C. M. Croce, L. Bolondi and M. Negrini: MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality. Clin Cancer Res, 15(16), 5073-81 (2009) DOI: 10.1158/1078-0432.CCR-09-0092

82. A. Tannapfel, D. Grund, A. Katalinic, D. Uhlmann, F. Kockerling, U. Haugwitz, M. Wasner, J. Hauss, K. Engeland and C. Wittekind: Decreased expression of p27 protein is associated with advanced tumor stage in hepatocellular carcinoma. Int J Cancer, 89(4), 350-5 (2000) DOI: 10.1002/1097-0215(20000720)89: 4<350::AID-IJC6>3.0.CO;2-3

83. Y. Ito, T. Takeda, M. Sakon, M. Tsujimoto, M. Monden and N. Matsuura: Expression of p57/Kip2 protein in hepatocellular carcinoma. Oncology, 61(3), 221-5 (2001) DOI: 10.1159/000055378

84. S. Nakai, T. Masaki, Y. Shiratori, T. Ohgi, A. Morishita, K. Kurokohchi, S. Watanabe and S. Kuriyama: Expression of p57(KIP2) in hepatocellular carcinoma: relationship between tumor differentiation and patient survival. Int J Oncol, 20(4), 769-75 (2002)

85. J. Li, Y. Wang, W. Yu, J. Chen and J. Luo: Expression of serum miR-221 in human hepatocellular carcinoma and its prognostic significance. Biochem Biophys Res Commun, 406(1), 70-3 (2011) DOI: 10.1016/j.bbrc.2011.01.111

86. J. Luo, M. Chen, H. Huang, T. Yuan, M. Zhang, K. Zhang and S. Deng: Circulating microRNA-122a as a diagnostic marker for hepatocellular carcinoma. Onco Targets Ther, 6, 577-83 (2013)

87. Y. Wang and C. G. Lee: Role of miR-224 in hepatocellular carcinoma: a tool for possible therapeutic intervention? Epigenomics, 3(2), 235-43 (2011) DOI: 10.2217/epi.11.5

88. Y. Ladeiro, G. Couchy, C. Balabaud, P. Bioulac-Sage, L. Pelletier, S. Rebouissou and J. Zucman-Rossi: MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. Hepatology, 47(6), 1955-63 (2008) DOI: 10.1002/hep.22256

89. Q. Li, G. Wang, J. L. Shan, Z. X. Yang, H. Z. Wang, J. Feng, J. J. Zhen, C. Chen, Z. M. Zhang, W. Xu, X. Z. Luo and D. Wang: MicroRNA-224 is upregulated in HepG2 cells and involved in cellular migration and invasion. J Gastroenterol Hepatol, 25(1), 164-71 (2010) DOI: 10.1111/j.1440-1746.2009.05971.x

90. Y. Wang, A. T. Lee, J. Z. Ma, J. Wang, J. Ren, Y. Yang, E. Tantoso, K. B. Li, L. L. Ooi, P. Tan and 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(19), 13205-15 (2008) DOI: 10.1074/jbc.M707629200

91. J. Secombe, S. B. Pierce and R. N. Eisenman: Myc: a weapon of mass destruction. Cell, 117(2), 153-6 (2004) DOI: 10.1016/S0092-8674(04)00336-8

92. J. Gui, Y. Tian, X. Wen, W. Zhang, P. Zhang, J. Gao, W. Run, L. Tian, X. Jia and Y. Gao: Serum microRNA characterization identifies miR-885-5p as a potential marker for detecting liver pathologies. Clin Sci (Lond), 120(5), 183-93 (2011) DOI: 10.1042/CS20100297

93. A. M. Liu, T. J. Yao, W. Wang, K. F. Wong, N. P. Lee, S. T. Fan, R. T. Poon, C. Gao and J. M. Luk: Circulating miR-15b and miR-130b in serum as potential markers

for detecting hepatocellular carcinoma: a retrospective cohort study. BMJ Open, 2(2), e000825 (2012)
DOI: 10.1136/bmjopen-2012-000825

94. Y. Yamamoto, N. Kosaka, M. Tanaka, F. Koizumi, Y. Kanai, T. Mizutani, Y. Murakami, M. Kuroda, A. Miyajima, T. Kato and T. Ochiya: MicroRNA-500 as a potential diagnostic marker for hepatocellular carcinoma. Biomarkers, 14(7), 529-38 (2009)
DOI: 10.3109/13547500903150771

95. C. T. Barry, M. D'Souza, M. McCall, S. Safadjou, C. Ryan, R. Kashyap, C. Marroquin, M. Orloff, A. Almudevar and T. E. Godfrey: Micro RNA expression profiles as adjunctive data to assess the risk of hepatocellular carcinoma recurrence after liver transplantation. Am J Transplant, 12(2), 428-37 (2012)
DOI: 10.1111/j.1600-6143.2011.03788.x

96. E. A. Afanasyeva, P. Mestdagh, C. Kumps, J. Vandesompele, V. Ehemann, J. Theissen, M. Fischer, M. Zapatka, B. Brors, L. Savelyeva, V. Sagulenko, F. Speleman, M. Schwab and F. Westermann: MicroRNA miR-885-5p targets CDK2 and MCM5, activates p53 and inhibits proliferation and survival. Cell Death Differ, 18(6), 974-84 (2011)
DOI: 10.1038/cdd.2010.164

97. L. Lin, Y. Lin, Y. Jin and C. Zheng: Microarray analysis of microRNA expression in liver cancer tissues and normal control. Gene, 523(2), 158-60 (2013)
DOI: 10.1016/j.gene.2013.02.055

98. A. Suetsugu, M. Nagaki, H. Aoki, T. Motohashi, T. Kunisada and H. Moriwaki: Characterization of CD133+ hepatocellular carcinoma cells as cancer stem/progenitor cells. Biochem Biophys Res Commun, 351(4), 820-4 (2006)
DOI: 10.1016/j.bbrc.2006.10.128

99. S. Ma, T. K. Lee, B. J. Zheng, K. W. Chan and X. Y. Guan: CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene, 27(12), 1749-58 (2008)
DOI: 10.1038/sj.onc.1210811

100. S. Ma, K. W. Chan, L. Hu, T. K. Lee, J. Y. Wo, I. O. Ng, B. J. Zheng and X. Y. Guan: Identification and characterization of tumorigenic liver cancer stem/progenitor cells. Gastroenterology, 132(7), 2542-56 (2007)
DOI: 10.1053/j.gastro.2007.04.025

101. S. Ma, 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 and 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(6), 694-707 (2010)
DOI: 10.1016/j.stem.2010.11.010

102. C. J. Guo, Q. Pan, D. G. Li, H. Sun and B. W. Liu: miR-15b and miR-16 are implicated in activation of the rat hepatic stellate cell: An essential role for apoptosis. J Hepatol, 50(4), 766-78 (2009)
DOI: 10.1016/j.jhep.2008.11.025

103. F. Meng, R. Henson, H. Wehbe-Janek, K. Ghoshal, S. T. Jacob and T. Patel: MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology, 133(2), 647-58 (2007)
DOI: 10.1053/j.gastro.2007.05.022

104. J. Xu, C. Wu, X. Che, L. Wang, D. Yu, T. Zhang, L. Huang, H. Li, W. Tan, C. Wang and D. Lin: Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. Mol Carcinog, 50(2), 136-42 (2011)
DOI: 10.1002/mc.20712

105. Q. W. Wong, R. W. Lung, P. T. Law, P. B. Lai, K. Y. Chan, K. F. To and N. Wong: MicroRNA-223 is commonly repressed in hepatocellular carcinoma and potentiates expression of Stathmin1. Gastroenterology, 135(1), 257-69 (2008)
DOI: 10.1053/j.gastro.2008.04.003

106. R. H. Yuan, Y. M. Jeng, H. L. Chen, P. L. Lai, H. W. Pan, F. J. Hsieh, C. Y. Lin, P. H. Lee and H. C. Hsu: Stathmin overexpression cooperates with p53 mutation and osteopontin overexpression, and is associated with tumour progression, early recurrence, and poor prognosis in hepatocellular carcinoma. J Pathol, 209(4), 549-58 (2006)
DOI: 10.1002/path.2011

107. J. Chang, E. Nicolas, D. Marks, C. Sander, A. Lerro, M. A. Buendia, C. Xu, W. S. Mason, T. Moloshok, R. Bort, K. S. Zaret and J. M. Taylor: miR-122, a mammalian liver-specific microRNA, is processed from hcr mRNA and may downregulate the high affinity cationic amino acid transporter CAT-1. RNA Biol, 1(2), 106-13 (2004)
DOI: 10.4161/rna.1.2.1066

108. H. Kutay, S. Bai, J. Datta, T. Motiwala, I. Pogribny, W. Frankel, S. T. Jacob and K. Ghoshal: Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. J Cell Biochem, 99(3), 671-8 (2006)
DOI: 10.1002/jcb.20982

109. H. Varnholt, U. Drebber, F. Schulze, I. Wedemeyer, P. Schirmacher, H. P. Dienes and M. Odenthal: MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. Hepatology, 47(4), 1223-32 (2008)
DOI: 10.1002/hep.22158

110. W. C. Tsai, 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 and A. P. Tsou: MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. Hepatology, 49(5), 1571-82 (2009)
DOI: 10.1002/hep.22806

111. S. Bai, M. W. Nasser, B. Wang, S. H. Hsu, J. Datta, H. Kutay, A. Yadav, G. Nuovo, P. Kumar and K. Ghoshal: MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. J Biol Chem, 284(46), 32015-27 (2009)
DOI: 10.1074/jbc.M109.016774

112. F. Fornari, L. Gramantieri, C. Giovannini, A. Veronese, M. Ferracin, S. Sabbioni, G. A. Calin, G. L. Grazi, C. M. Croce, S. Tavolari, P. Chieco, M. Negrini and L. Bolondi: MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res, 69(14), 5761-7 (2009)
DOI: 10.1158/0008-5472.CAN-08-4797

113. C. Coulouarn, V. M. Factor, J. B. Andersen, M. E. Durkin and S. S. Thorgeirsson: Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene, 28(40), 3526-36 (2009)
DOI: 10.1038/onc.2009.211

114. A. J. van der Meer, W. R. Farid, M. J. Sonneveld, P. E. de Ruiter, A. Boonstra, A. J. van Vuuren, J. Verheij, B. E. Hansen, R. J. de Knegt, L. J. van der Laan and H. L. Janssen: Sensitive detection of hepatocellular injury in chronic hepatitis C patients with circulating hepatocyte-derived microRNA-122. J Viral Hepat, 20(3), 158-66 (2013)
DOI: 10.1111/jvh.12001

115. H. Yamada, K. Suzuki, N. Ichino, Y. Ando, A. Sawada, K. Osakabe, K. Sugimoto, K. Ohashi, R. Teradaira, T. Inoue, N. Hamajima and S. Hashimoto: Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. Clin Chim Acta, 424, 99-103 (2013)
DOI: 10.1016/j.cca.2013.05.021

116. O. F. Laterza, L. Lim, P. W. Garrett-Engele, K. Vlasakova, N. Muniappa, W. K. Tanaka, J. M. Johnson, J. F. Sina, T. L. Fare, F. D. Sistare and W. E. Glaab: Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. Clin Chem, 55(11), 1977-83 (2009)
DOI: 10.1373/clinchem.2009.131797

117. Q. Liu, H. Fu, F. Sun, H. Zhang, Y. Tie, J. Zhu, R. Xing, Z. Sun and X. Zheng: miR-16 family induces cell cycle arrest by regulating multiple cell cycle genes. Nucleic Acids Res, 36(16), 5391-404 (2008)
DOI: 10.1093/nar/gkn522

118. C. J. Guo, Q. Pan, B. Jiang, G. Y. Chen and D. G. Li: Effects of upregulated expression of microRNA-16 on biological properties of culture-activated hepatic stellate cells. Apoptosis, 14(11), 1331-40 (2009)
DOI: 10.1007/s10495-009-0401-3

119. K. Z. Qu, K. Zhang, H. Li, N. H. Afdhal and M. Albitar: Circulating microRNAs as biomarkers for hepatocellular carcinoma. J Clin Gastroenterol, 45(4), 355-60 (2011)
DOI: 10.1097/MCG.0b013e3181f18ac2

120. T. Xu, Y. Zhu, Y. Xiong, Y. Y. Ge, J. P. Yun and S. M. Zhuang: MicroRNA-195 suppresses tumorigenicity and regulates G1/S transition of human hepatocellular carcinoma cells. Hepatology, 50(1), 113-21 (2009)
DOI: 10.1002/hep.22919

121. F. Fornari, M. Milazzo, P. Chieco, M. Negrini, G. A. Calin, G. L. Grazi, D. Pollutri, C. M. Croce, L. Bolondi and L. Gramantieri: MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res, 70(12), 5184-93 (2010)
DOI: 10.1158/0008-5472.CAN-10-0145

122. Q. H. Xie, X. X. He, Y. Chang, S. Z. Sun, X. Jiang, P. Y. Li and J. S. Lin: MiR-192 inhibits nucleotide excision repair by targeting ERCC3 and ERCC4 in HepG2.2.15 cells. Biochem Biophys Res Commun, 410(3), 440-5 (2011)
DOI: 10.1016/j.bbrc.2011.05.153

123. W. Li, 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 and J. Gu: Diagnostic and prognostic implications of microRNAs in human hepatocellular carcinoma. Int J Cancer, 123(7), 1616-22 (2008)
DOI: 10.1002/ijc.23693

124. F. Petrocca, R. Visone, M. R. Onelli, M. H. Shah, M. S. Nicoloso, I. de Martino, D. Iliopoulos, E. Pilozzi, C. G. Liu, M. Negrini, L. Cavazzini, S. Volinia, H. Alder, L. P. Ruco, G. Baldassarre, C. M. Croce and A. Vecchione: E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. Cancer Cell, 13(3), 272-86 (2008)
DOI: 10.1016/j.ccr.2008.02.013

125. S. Ambs, R. L. Prueitt, M. Yi, R. S. Hudson, T. M. Howe, F. Petrocca, T. A. Wallace, C. G. Liu, S. Volinia, G. A. Calin, H. G. Yfantis, R. M. Stephens and C. M. Croce: Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. Cancer Res, 68(15), 6162-70 (2008)
DOI: 10.1158/0008-5472.CAN-08-0144

126. L. O'Connor, A. Strasser, L. A. O'Reilly, G. Hausmann, J. M. Adams, S. Cory and D. C. Huang: Bim: a novel member of the Bcl-2 family that promotes apoptosis. EMBO J, 17(2), 384-95 (1998)
DOI: 10.1093/emboj/17.2.384

127. M. Z. Xu, T. J. Yao, N. P. Lee, I. O. Ng, Y. T. Chan, L. Zender, S. W. Lowe, R. T. Poon and J. M. Luk: Yes-associated protein is an independent prognostic marker in hepatocellular carcinoma. Cancer, 115(19), 4576-85 (2009)
DOI: 10.1002/cncr.24495

128. A. M. Liu, R. T. Poon and J. M. Luk: MicroRNA-375 targets Hippo-signaling effector YAP in liver cancer and inhibits tumor properties. Biochem Biophys Res Commun, 394(3), 623-7 (2010)
DOI: 10.1016/j.bbrc.2010.03.036

129. X. X. He, Y. Chang, F. Y. Meng, M. Y. Wang, Q. H. Xie, F. Tang, P. Y. Li, Y. H. Song and J. S. Lin: MicroRNA-375 targets AEG-1 in hepatocellular carcinoma and suppresses liver cancer cell growth in vitro and in vivo. Oncogene, 31(28), 3357-69 (2012)
DOI: 10.1038/onc.2011.500

130. B. K. Yoo, L. Emdad, Z. Z. Su, A. Villanueva, D. Y. Chiang, N. D. Mukhopadhyay, A. S. Mills, S. Waxman, R. A. Fisher, J. M. Llovet, P. B. Fisher and D. Sarkar: Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression. J Clin Invest, 119(3), 465-77 (2009)
DOI: 10.1172/JCI36460

131. B. J. Reinhart, F. J. Slack, M. Basson, A. E. Pasquinelli, J. C. Bettinger, A. E. Rougvie, H. R. Horvitz and G. Ruvkun: The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature, 403(6772), 901-6 (2000)
DOI: 10.1038/35002607

132. A. E. Pasquinelli, B. J. Reinhart, F. Slack, M. Q. Martindale, M. I. Kuroda, B. Maller, D. C. Hayward, E. E. Ball, B. Degnan, P. Muller, J. Spring, A. Srinivasan, M. Fishman, J. Finnerty, J. Corbo, M. Levine, P. Leahy, E. Davidson and G. Ruvkun: Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature, 408(6808), 86-9 (2000)
DOI: 10.1038/35040556

133. A. Esquela-Kerscher and F. J. Slack: Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer, 6(4), 259-69 (2006)
DOI: 10.1038/nrc1840

134. A. E. Pasquinelli, A. McCoy, E. Jimenez, E. Salo, G. Ruvkun, M. Q. Martindale and J. Baguna: Expression of the 22 nucleotide let-7 heterochronic RNA throughout the Metazoa: a role in life history evolution? Evol Dev, 5(4), 372-8 (2003)
DOI: 10.1046/j.1525-142X.2003.03044.x

135. I. Bussing, F. J. Slack and H. Grosshans: let-7 microRNAs in development, stem cells and cancer. Trends Mol Med, 14(9), 400-9 (2008)
DOI: 10.1016/j.molmed.2008.07.001

136. S. M. Johnson, H. Grosshans, J. Shingara, M. Byrom, R. Jarvis, A. Cheng, E. Labourier, K. L. Reinert, D. Brown and F. J. Slack: RAS is regulated by the let-7 microRNA family. Cell, 120(5), 635-47 (2005)
DOI: 10.1016/j.cell.2005.01.014

137. J. Takamizawa, H. Konishi, K. Yanagisawa, S. Tomida, H. Osada, H. Endoh, T. Harano, Y. Yatabe, M. Nagino, Y. Nimura, T. Mitsudomi and T. Takahashi: Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res, 64(11), 3753-6 (2004)
DOI: 10.1158/0008-5472.CAN-04-0637

138. L. F. Sempere, M. Christensen, A. Silahtaroglu, M. Bak, C. V. Heath, G. Schwartz, W. Wells, S. Kauppinen and C. N. Cole: Altered MicroRNA expression confined to specific epithelial cell subpopulations in breast cancer. Cancer Res, 67(24), 11612-20 (2007)
DOI: 10.1158/0008-5472.CAN-07-5019

139. S. Mi, J. Lu, M. Sun, Z. Li, H. Zhang, M. B. Neilly, Y. Wang, Z. Qian, J. Jin, Y. Zhang, S. K. Bohlander, M. M. Le Beau, R. A. Larson, T. R. Golub, J. D. Rowley and J. Chen: MicroRNA expression signatures accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia. Proc Natl Acad Sci U S A, 104(50), 19971-6 (2007)
DOI: 10.1073/pnas.0709313104

140. S. Shimizu, T. Takehara, H. Hikita, T. Kodama, T. Miyagi, A. Hosui, T. Tatsumi, H. Ishida, T. Noda, H. Nagano, Y. Doki, M. Mori and N. Hayashi: The let-7 family of microRNAs inhibits Bcl-xL expression and potentiates sorafenib-induced apoptosis in human hepatocellular carcinoma. J Hepatol, 52(5), 698-704 (2010)
DOI: 10.1016/j.jhep.2009.12.024

141. J. Torrisani, B. Bournet, M. C. du Rieu, M. Bouisson, A. Souque, J. Escourrou, L. Buscail and P. Cordelier: let-7 MicroRNA transfer in pancreatic cancer-derived cells inhibits in vitro cell proliferation but fails to alter tumor progression. Hum Gene Ther, 20(8), 831-44 (2009)
DOI: 10.1089/hum.2008.134

142. P. Shapiro: Ras-MAP kinase signaling pathways and control of cell proliferation: relevance to cancer therapy. Crit Rev Clin Lab Sci, 39(4-5), 285-330 (2002) DOI: 10.1080/10408360290795538

143. J. L. Bos: ras oncogenes in human cancer: a review. Cancer Res, 49(17), 4682-9 (1989)

144. S. Liang, L. He, X. Zhao, Y. Miao, Y. Gu, C. Guo, Z. Xue, W. Dou, F. Hu, K. Wu, Y. Nie and D. Fan: MicroRNA let-7f inhibits tumor invasion and metastasis by targeting MYH9 in human gastric cancer. PLoS One, 6(4), e18409 (2011) DOI: 10.1371/journal.pone.0018409

145. N. B. Tsui, E. K. Ng and Y. M. Lo: Stability of endogenous and added RNA in blood specimens, serum, and plasma. Clin Chem, 48(10), 1647-53 (2002)

Abbreviations: ADAM: A disintegrin and metalloprotease; AEG-1: Astrocyte elevated gene-1; AFP: Alpha-fetoprotein; AFP-L3: AFP Lens culinaris agglutinin-reactive fraction; API-5: Apoptosis inhibitor 5; AUC: Area under the operating characteristic curve; CD1: Cyclin D1; CDK2: Cyclin-dependent kinase 2; CDK6: Cyclin-dependent kinase 6, CTC: Circulating tumor cell, DCP: Des-gamma-carboxyprothrombin; EMT: Epithelial to mesenchymal transition; ERα: Estrogen receptor alpha; ESR1: Estrogen receptor 1; HBV: Hepatitis B virus; HCC: Hepatocellular Carcinoma; HCV: Hepatitis C virus; HSC: Hepatic stellate cells; IGF-1: Insulin like growth factor 1; IGF1R: Insulin-like growth factor 1 receptor; Let-7: Lethal-7; MCM5: mini-chromosome maintenance protein; MCM7: minichromosome maintenance protein 7; mTOR: Mammalian target of rapamycin; PIAS3: Protein Inhibitor of Activated Signal Transducer and Activator of Transcription 3; PTEN: Phosphatase and tensin homolog; SRF: Serum response factor; STAT: Signal transducer and activator of transcription; STAT3: Signal Transducer and Activator of Transcription 3; STMN1: Stathmin 1; VEGF: Vascular endothelial growth factor; YAP: Yes-associated protein.

Key Words: MicroRNA, Serum microRNA, Circulating microRNA, Hepatocellular carcinoma, HCC, Prognosis, Diagnosis, Therapy, Treatment, Review

Send correspondence to: Christopher Christophi, The University of Melbourne, Department of Surgery Austin Health Studley Road, Heidelberg, Victoria, Australia 3084. Tel: 613-9496-5468, Fax: 613-945-8650, E-mail: c.christophi@unimelb.edu.au