MicroRNA Expression Profiles of Circulating Microvesicles in Hepatocellular Carcinoma

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Abstract

Background : **Microvesicles (MVs) packaged with microribonucleic acids (miRNAs) have been shown to be released mainly from tumor cells. However, little information is known for miRNAs from MVs in hepatocellular carcinoma (HCC). Hence, we explored the MVs' miRNAs expression profiles in HCC.**

Methods : **MVs were collected from peripheral blood of HCC, chronic hepatitis B (CHB) and normal controls. miRNA from MVs were labeled and analyzed by Agilent miRNA microarry. Bioinformatics tools were used to analyze function of the differentially expressed MVs' miRNAs.**

Results : **A total of 242 aberrantly expressed miRNAs were identified in HCC-MVs compared with CHB-MVs and the control. Among them, 115 miRNAs were over-expressed with up to 31 fold difference (miR-671-5p) and 127 were down-expressed with up to 0.041 fold difference (miR-432) in HCC. By software miRror2.0, nucleolar protein 3 (NOL3) was found to be the core player among 300 target genes of top ten up-regulated miRNAs and serine/arginine repetitive matrix 1(SRRM1) was central among the 219 targets of the top ten down-regulated miRNAs. We also analyzed GO categories for these predicted genes : cellular component, biological processes, and molecular function. The deregulation of MVs' miRNAs and their target genes were closely involved in the pathways of HCC.**

Conclusions : **Our study firstly demonstrated that miRNAs were differentially expressed in HCC-MVs compared with CHB and normal controls. Aberrant HCC-MVs miRNAs may play important roles in the development of HCC.** (Acta gastroenterol. belg., **2013**, 76, **386-392**).

Key words : hepatocellular carcinoma (HCC), microvesicles (MVs), microRNAs (miRNAs), target genes.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world. The annual number of new cases of HCC is over one million in the world, making it the 3rd leading cause of cancer-related death and the 5th of most common cancers (1,2). Chronic active hepatitis is considered as an important risk factor for HCC occurrence, especially chronic HBV infection, and this also accounts for the high risk of HCC in patients with active cirrhosis. In early stage, we usually diagnose HCC with imaging and serum markers for most patients. However, serum alpha fetoprotein (AFP) is not sufficient enough as a diagnostic marker because of the low sensitivity and frequent false-positive results, though it is a well-established biochemical parameter for HCC. And the sensitivity of various imaging detection techniques depend on the size of the lump (3). Small lump is easily neglected by routine examination. Furthermore, asymptomatic HCC patients usually lack awareness of treatment and screening in early stage (4). Therefore, about 80% of patients are usually diagnosed in advanced stage and subsequently lose the chance of surgical treatment.

Microvesicle (MVs) are secretory particles released by various cell types. MVs with the diameters of 30-200 nm have long been regarded as "cellular dust". When they were first described by Wolf in 1967 (5). But recently, studies have shown that MVs can contain nucleic acids, proteins and bioactive molecules (6) and therefore play important roll in cell signaling and cell communication. The function of MVs is closely related with their contents. MVs, as carriers of molecular information in relation to cancer progression and metastasis, can carry bioactive molecules, including growth factors and their receptors, proteases, adhesion molecules, signaling molecules, as well as DNA, mRNA and miRNA, and they together constitute the 'signaling complex' (7). MVs can modulate the interaction of tumor cells with inflammatory, immune, stromal signals and start the formation of the metastatic niche in tumor microenvironments (8). Therefore, MVs are now recognized as important mediators of intercellular communication rather than irrelevant cell debris.

miRNAs are small non-coding regulatory RNAs that play important roles in the regulation of gene expression at the post-transcriptional level by targeting mRNAs for degradation and inhibiting protein translation. miRNAs

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Submission date : 08/01/2013
Acceptance date : 01/04/2013

play roles in mRNA degradation and inhibition of protein translation through incomplete pairing with 3'-UTR of the mRNA, that negatively regulate gene expression. miRNAs are widely involved in differentiation, migration, apoptosis, tumor formation, cell proliferation and other physiological pathology process through regulation of downstream target gene mRNA (9). It was first reported that aberrant miRNA expression was related to the origin and differentiation of the tumor in 2005, indicating the potential of miRNAs in cancer diagnosis (10). Circulating miRNAs were emerging as novel noninvasive biomarkers for the early detection and prognosis of HCC (11). MVs packaged with miRNAs are released mainly from progenitors of differentiated cells and tumor cells (6), but little information is available for HCC. Our study is aimed to explore circulating MVs' miRNAs expression profiles in peripheral blood of HCC patients and analyze their roles in the development of HCC. This is, to our knowledge, for the first time a comprehensive miRNA expression profile has been made for MVs derived from HCC patients. Our study will shed light on the possible roles of MVs in HCC disease progression.

Methods

Sample collection

The samples used in this study were from chronic hepatitis B (CHB), HCC patients and normal healthy adults. All patients were admitted to Wuhan Union Hospital, Tongji Medical College, Huazhong University of Science and Technology during the period from May to September in 2011. CHB diagnosis without cirrhosis was based on hepatitis B virus markers (HBVM) and HBV-DNA quantitation. The HCC patients without cirrhosis were diagnosed according to imaging, histopathology and AFP, following "the standard of clinical diagnosis and stage with hepatocellular carcinoma" amended by the Chinese Society of Liver Cancer in 2001. In addition, HCC patients had a history of HBV infection and the serum viral load was consecutively high (more than 10^4 copies/mL). Healthy volunteers were normal controls who had no diseases via health examination and no treatment with any drugs prior to and during the investigation phase. The patients and the volunteers signed informed consents. Our study was approved by the ethic committee of Wuhan Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. All samples were divided into three groups and the patients' general medical information was provided in Table 1.

Isolation of MVs from the samples

Blood samples were collected in EDTA-containing tubes. The plasma was isolated from the whole blood on ice. The samples were centrifuged at 2,000 g for 20 minutes at 4°C to completely remove cellular debris, and then MVs were purified by ultracentrifugation at 16,000 g for 1 hour at 4°C with collected supernatants (12). MVs pellets were resuspended in lysate and then stored at -80°C until use. The Jeol 1210 transmission electron microscope was used to examine the sections.

RNA extraction, purification and miRNA microarray

The total RNA was extracted from acquired MVs with the mirVana™ miRNA Isolation Kit (Cat#AM1560, Ambion, Austin, TX, US) following manufacturer's protocols. miRNA in total RNA was labeled with miRNA Complete Labeling and Hyb Kit (Cat#5190-0456, Agilent technologies, Santa Clara). Each slide was hybridized with 100 ng Cy3-labeled RNA according to the manufacturer's instructions. Human microRNA microarrays from Agilent Technologies which contain probes for 888 human microRNAs from the Sanger database v.12.0. were used. Agilent Microarray Scanner (Cat#G2565BA, Agilent technologies, Santa Clara) were used to scan the microarray images and the acquired images were analyzed by Feature Extraction software 10.7 (Agilent technologies, Santa Clara).

Quantitative RT-PCR of human miRNA

Quantitative RT-PCR (qRT-PCR) analysis was performed to detect the level of miRNAs. cDNA was produced using the miScript Reverse Transcription Kit. And then, the cDNA served as the template for real-time PCR analysis using the miScript Primer Assay in combination with the miScript SYBR Green PCR Kit. miRNAs were amplified using the miScript Universal Primers.

Bioinformatics analysis

In order to improve the accuracy of the target gene prediction, we used several databases to predict at the same time and selected the overlapped target genes as candidates. The comprehensive target gene prediction software miRror2.0 was used. Minimum 8 databases

Table 1. — **Primary clinical information of patients**

Group	number	sex		Age		Metastasis
		F	M	Median	Range	
Control	205	102	103	42	17-62	–
CHB	354	98	256	38	15-58	–
HCC+CHB	265	27	238	49	26-81	68

combination were as follows : TargetRank-all (TRnkall), TargetRank-conserved (TRnkcon), miRDB, microcosm (mCosm), miRandaConserved (microRNA.org), PITA-TOP, DIANA-microT (microT), EIMMO-MirZ (MirZ). They were selected commonly. Each database had a system to score and rank for each target genes, and the criteria $p < 0.05$ was used as a quality threshold for gene selection. These candidate target genes were predicted in at least two databases and they were regulated by at least two miRNAs.

Gene Ontology (GO) annotation can analyze the functional classification of all target genes. After gene ontology analysis, we further analyzed the signaling pathways that the target genes might be involved using DIAY software.

Results

MVs' miRNAs expression profiles in HCC patients

Under the electron microscope, the diameter of MV was less than 1 μm and there was no significant difference in morphology between MV derived from the peripheral blood of CHB and HCC patients and normal controls. However, when comparing the MVs' miRNAs expression profiles of CHB and HCC patients and normal controls, we found significant difference. A total of 706 MVs' miRNAs were detected in CHB, HCC and normal controls. Among them, there were 351 miRNAs detected in CHB-MVs, 385 miRNAs in HCC-MVs and 456 MVs' miRNAs in normal controls. 301 MVs' miRNAs were co-expressed in all three groups. We found 242 MVs' miRNAs aberrantly expressed through comparing the chip miRNA probe signal intensity between three groups. While 115 and 127 MVs' miRNAs displayed up regulation or down regulation, respectively, which were identified by expression levels of more than 2-fold difference or less than 0.5-fold difference in the microarray results. The ten most up-regulated and the ten most down-regulated MVs' miRNAs were listed in Table 2. The most over-represented miRNA was miR-671-5p with a fold difference of 31.28. miR-432 was the lowest with a fold difference of 0.04.

Target gene analysis of MVs' miRNAs in HCC patients

We looked up the ten up-regulated miRNAs in table 1 with online software miRror2.0 to analyze their target genes in HCC patients and got 300 candidate target genes accords with the above conditions. Nucleolar protein 3 (NOL3) was found to be the core player among the 300 target genes. These genes were predicted in more than 4 databases : carboxy-terminal domain (CTD), hexosaminidase A (HEXA), SH3 and multiple ankyrin repeat domains 3 (SHANK3) , CREB regulated transcription coactivator 1 (CRTC1), parathymosin(PTMS), zer-1 homolog (ZER1), cerebellar degeneration-related protein 2-like (CDR2L), WD and tetratricopeptide repeats 1 (WDTC1). Similarly, we predicted 219 genes of the ten down regulated MVs' miRNAs in table 1. Serine/arginine repetitive matrix 1 (SRRM1) was central among them. These genes were also more databases : ubiquitin-conjugating enzyme E2Q family member 1 (UBE2Q1), centrosomal protein 76 (CEP76), amyloid beta(A4) precursor protein (APP), myosin IB (MYO1B), member of nuclear receptor subfamily 2 (NR2F2), eukaryotic translation initiation factor 4 gamma 3 (EIF4G3), small nuclear ribonucleoprotein 40 (SNRNP40), protein phosphatase 3 catalytic subunit alpha isozyme (PPP3CA), semaphorin 3c (SEMA3C), zinc finger CCCH-type containing 7A (ZC3H7A), transmembrane 4 L six family member 1(TM4SF1), zinc finger protein 318 (ZNF318), ubiquitin specific peptidase 34 (USP34), maternal embryonic leucine zipper kinase (MELK), R3H domain containing 2 (R3HDM2), inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK), HD domain containing 2 (HDDC2), platelet derived growth factor C (PDGFC), CDKN2A interacting protein (CDKN2AIP), membrane-associated ring finger 1 (MARCH1), lin-7 homolog C(LIN7C), retinoic acid induced 2(RAI2), heat shock 70kDa protein 2 (HSPA2). However, CTD, CRTC1, PTMS, ZER1, CDR2L, WDTC1, UBE2Q1, CEP76, EIF4G3, SEMA3C, ZC3H7A, ZNF318, USP34, MELK, R3HDM2, HDDC2 and CDKN2AIP had no detailed comments in addition to basic information (ID, chromosome location, sequence).

Table 2. — **The list of ten most up- and down-expressed MVs' miRNAs derived from HCC patients**

miRNA name	Foldchange	miRNA name	Foldchange
miR-432	0.041	miR-671-5p	31.282
miR-154*	0.050	miR-1290	27.391
miR-27-3p	0.057	miR-150*	19.286
miR-487b	0.084	miR-939	14.736
miR-323-3p	0.088	miR-574-5p	14.142
miR-487a	0.095	miR-1249	12.999
miR-379	0.097	miR-762	12.161
miR-382	0.097	miR-940	12.123
miR-381	0.098	miR-198	12.040
miR-495	0.100	miR-1234	11.237

Table 3. — **The subclass enrichment for target genens of 10 up-regulated MVs' miRNAs**

GO ID	GO Term	Hits
Biological process		
GO:0051252	Regulation of RNA metabolic process	28
GO:0006355	Regulation of transcription, DNA-dependent	27
GO:0035556	Intracellular signal transduction	23
GO:0006357	Regulation of transcription from RNA polymerase ll promoter	15
GO:0042592	Homeostatic process	14
GO:0016192	Vesicle-mediated transport	13
Molecular function		
GO:0030528	Transcription regulator activity	25
GO:0003743	Transcription initiation factor activity	19
GO:0019904	Protein domain specific binding	10
GO:0031420	Alkali metal ion binding	7
GO:0005543	Phospholipid binding	6
GO:0019905	Syntaxin binding	3
Cellular component		
GO:0005886	Plasma membrane	48
GO:0044459	Plasma membrane part	35
GO:0005829	cytosol	21
GO:0000267	Cell fraction	20
GO:0031982	Vesicle	17
GO:0031410	Cytoplasmic vesicle	16

Gene Ontology (GO) analysis

We also found 426 GO classifications for 300 target genes of ten up regulated miRNAs in table 1. There were 149 biological process (BP) sub-types, 144 molecular-function (MF) sub-types, 133 cellular component (CC) sub-types. We calculated the enrichment value separately for the subclasses in the classes and $p < 0.05$ was significant. BP had 26 subclasses enrichment and the most was small GTPase mediated signal transduction (GO :0007264). Analogously, MF contained 8 subclasses enrichment and the most was syntaxin binding (GO :0031420). CC included 20 subclasses enrichment and vesicle (GO :0031982) was the most. We compiled a list with the three categories in which each gene is involved for target genes of 10 up-regulated MVs' miRNAs, and then cross-referencing all those categories to determine the number of genes is in each category. Table 3 showed the top six subclasses enrichment of BP, MF, and CC according to the number of target genes.

We got 408 GO classifications for 219 target genes of ten down regulated MVs' miRNAs in table 1. There were 147 BP sub-types, 138 MF sub-types and 123 CC sub-types. BP had 69 subclasses enrichment and the most was RNA processing (GO :0006396). Similarly, MF contained 6 subclasses enrichment and the most was RNA binding (GO :0003723). CC included 17 subclasses enrichment and membrane-enclosed lumen (GO :0031974) was the most. We also compiled a list with the three categories in which each gene is involved for target genes of 10 down-regulated MVs' miRNAs, and then count the number of genes in each category. The list of the top six subclasses enrichment of BP, MF, and CC according to the number of genes was shown in Table 4.

Regulation of signaling pathway in HCC patients

We found that the target genes by MVs' miRNAs involved in many signaling pathways when we analyzed pathways of aberrant MVs' miRNAs with KEGG software. The activation and inactivation of certain intracellular signaling pathways play critical roles in the cancer development. To seek the signaling pathways involved in HCC derived MVs' miRNAs, we further analyzed the 3523 target genes targeted by MVs' miRNAs. We found that there were mainly 19 different pathways (Table 5) in HCC. They were mainly related to immune reaction, metabolism, tumor suppressive and cancerous activity, proliferation regulation, apoptosis and basic biological process.

Discussion

It is well known that the expression of MVs is elevated, whereas miRNAs and their key targets are either elevated or reduced in almost all types of cancer. Our current study is a comprehensive analysis of differential expression of MVs' miRNAs and their targeted genes in HCC. The significant observation from our study is the deregulation of MVs' miRNAs and their targeted genes in the development of HCC.

Our study showed that the expression profile of MVs' miRNAs from HCC peripheral blood changed obviously when compared with CHB and normal controls. There were 115 up-regulated and 127 down-regulated miRNAs among 242 aberrant MVs' miRNAs in HCC and down-regulated miRNAs were more than up-regulated miRNAs. Lots of literature have reported that the majority of abnormal miRNAs are down-regulated in tumor

Table 4. — **The subclass enrichment for target genes of 10 down-regulated MVs' miRNAs**

GO ID	GO Term	Hits
Biological process		
GO:0007049	Cell cycle	20
GO:0006396	RNA processing	20
GO:0006508	proteolysis	18
GO:0031328	Positive regulation of cellular biosynthetic process	16
GO:0065003	Macromolecular complex assembly	16
GO:0022402	Cell cycle process	16
Molecular function		
GO:0003723	RNA binding	20
GO:0005496	Steroid binding	4
GO:0030165	PDZ domain binding	3
GO:0000375	RNA splicing, via transesterification reactions	3
GO:0030957	TAT protein binding	2
GO:0005499	Vitamin D binding	2
Cellular component		
GO:0031974	Membrane-enclosed lumen	33
GO:0043233	Organelle lumen	32
GO:0070013	Intracellular organelle lumen	30
GO:0031981	Nuclear lumen	27
GO:0005654	nucleoplasm	20
GO:0044451	Nucleoplasm part	13

patients (13). In our study, miR-16 was decreased in serum. Moreover, miR-16 was more sensitive as HCC detection marker than AFP and was significantly correlated with HCC (14). A few altered miRNAs can behave oncogenic or having tumor suppressor properties that either induce progression of a cell to cancer or prevent cell progression to cancer, respectively, so they are also called oncomirs and anti-oncomirs. It has been reported that oncomirs-miR-21 up regulation is linked directly to HCC (15). Signal transducer and activator of transcription 3 (STAT3) is able to bind the promoter of the miR-21 primary transcript, then leading to its activation (13), explaining the high expression of miR-21 in HCC. In addition, overexpression of a subset of miRNAs has been inversely correlated with certain tumor phenotypes, suggesting they play roles in tumor suppression (16). These anti-oncomirs can act as natural inhibitors of oncogene function, indicating the possibility that they might be developed as novel therapeutics.

Target genes of MVs' miRNAs in HCC will be useful molecular markers for the genesis and progression of HCC. An intimate analysis of the putative gene targets of these miRNAs will shed light on the potential implications of MVs in HCC microenvironment. It had been reported that annexin A2 (ANXA2) might be a novel marker for tumor angiogenesis in HCC (17). In our study, ANXA2 was found to be a potential target of miR-1. In addition, the studies are not clear about those genes without detailed comments (for example : CTD, CRTC1, PTMS, ZER1). But in vitro treatment of HCC cells with ibulocydine could rapidly block phosphorylation of the CTD of the large subunit of RNA polymerase II (18). The CTD targeted by miR-486-5p in HCC-MVs might have potential values in the clinical management of HCC patients. The aberrant activation of CRTCs was linked with oncogenic activities (19). CRTC2 regulated by miR-149 in HCC-MVs might participate in some oncogenic activities in the diverse physiological and developmental processes of HCC. IBTK was targeted by MVs' miR-92b, which bound Bruton's tyrosine kinase (BTK) to lower its activity, disrupt the flow of BTK-mediated calcium and inhibit NF-κB signal. However, the roles and mechanisms of aforesaid genes in the pathogenesis of HCC had not been previously investigated.

We also analyzed GO categories : cellular component, biological processes, and molecular function as well as biochemical function and cellular role**.** The GO database was not only used for the identification of disease-relevant biological processes of the differentially expressed genes but also for the functional annotation (20). These terms were characterized by very pleiotropic functions affecting BP throughout the whole process of HCC. These genes represented BP that comprised regulation of RNA metabolic process, intracellular signal transduction and so on (listed in table 2 and table 3). MF represented activities of the molecular level, such as binding activities. In our study, it appeared to involve molecular transcription and kinds of binding activities. CC described locations for subcellular structures and macromolecular complexes. CC contained the majority of putative disorder-containing proteins that provided some protection from proteolysis such as the cell cortex and nucleus (21). Using GO annotations, Patil MA *et al*. (22) found a series of genes that encode secreted (e.g. GPC3, LCN2, and DKK1) or membrane-bound proteins (e.g. GPC3, IGSF1, and PSK-1), which may be of value for the diagnosis of

Table 5. — **Target genes related signaling pathways in HCC**

Signaling pathway	Target genes	Mainly MVs' miRNAs
MAPK signaling pathway	MAP3K1,BDNE,CRKL,STK4,NF1	let-7c,miR-128,miR-23a
TGF-βsignaling pathway	SMAD2,ACVR2B,BMPR2	miR-128,miR-194
Wnt signaling pathway	SMAD2,MAP3K7	miR-128,miR-377
Jak-STAT signaling pathway	LIFR,CBL,IL-2,SOCS3	miR-27b,miR-425,miR-150
ErbB signaling pathway	CBL,CRKL	miR-425,miR-150
P53 signaling pathway	CDK6, CCND1	miR-550a,mi-139b
GnRH signaling pathway	MAP3K1	let-7c
Insulin signaling pathway	CBL,CRKL	miR-425,miR-150
T cell receptor	CBL,SOS	miR-425,miR-150,miR-1202
Cell cycle signaling pathway	CDK6,SMAD2	miR-550a, miR-128
Adherens junction	IGF1R,SMAD2,SSX2IP	miR-128, miR-425,miR-1275
Cell Cycle G1S Check Point	CDK6,CDKN1B, TFDP1	miR-550a,miR-377,miR-24
NF-kB Signaling Pathway	TNFAIP3, MAP3K1, MAP3K7	mi-1229, let-7c,miR-377
E2F1 Destruction Pathway	CDC34, TFDP1	miR-30b, let-7c
IL 2 signaling pathway	GRB2, JAK1, SOS1	miR-1202,miR-27b,miR-106b
Toll-Like Receptor Pathway	MAP3K1, MAP3K7, MAPK14	let-7c, miR-377,miR-128
Ras Signaling Pathway	PIK3R1, RAC1,RASSF2	miR-486-5p, miR-150,miR-659
Rho cell motility signaling pathway	PIP5K1B	miR-27b
IGF Signaling Pathway	IGF1, IGF2BP2, IGF2BP3, IGFBP3	mi-425,miR-9,miR-1275

HCC. A significant enrichment of genes and function on the basis of GO annotations made it possible to indicate putative functions of MVs' miRNAs in HCC. The functional profile will be of significance for further studies for HCC.

HCC is a heterogeneous tumor that develops via the deregulation of multiple signaling pathways and molecular alterations. In our assay, pathway annotation applying to the target gene pool showed that the genes targeted by the aberrantly expressed MVs' miRNAs were mostly involved in signal pathways that regulated proliferation, apoptosis and immune reactions in HCC. A recent study reported that the activation of IGF signaling in HCC was significantly associated with mTOR signaling (22). Integrative genomic analysis showed enrichment of activation of IGF signaling in the proliferation subclass of HCC (23). The pathway named by Cell Cycle G_1S Check Point which was critical in cell cycle process may cause inappropriate proliferation of HCC cells (24). Over-expression of miR-122 inhibits HCC cell growth and promotes cell apoptosis by affecting Wnt/β-catenin-TCF signaling pathway (25). Ras and Jak/Stat pathways in HCC are also ubiquitously activated (26). The genes targeted by MVs' miR-381 were involved in mTOR signaling, TGF-ß signaling, cytokine and receptor signaling. Therefore, we speculated that the miRNAs in MVs may play their roles through the signaling pathways of targeted genes in HCC.

In conclusion, our study demonstrated for the first time that miRNAs are differentially expressed in MVs derived from HCC patients in comparison to CHB and normal controls. Our result also indicated that the aberrant HCC-MVs miRNAs may play an important role in the progression of HCC by deregulation of HCC related gene expression and HCC related signaling pathways. Our study will be helpful to better understand HCC and the microenvironment.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant 81170462). We would like to thank the teachers at the Center for Stem Cell Research and Application for technical assistance and the professors at clinical laboratory in the Department of Infectious Disease for their help in collecting samples.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose the conflict of interest with this manuscript.

References

1. BOSCH F.X., RIBES J., BORRAS J. Epidemiology of primary liver cancer. *Semin. Liver Dis*., 1999, **19** : 271–285.
2. ZHOU L., WANG C.P., LU Y.Y., BAI W.L., QU J.H., LOU M. *et al*. Decrease in size of non-treated lesions after cryoablation for hepatocellular carcinoma. *Hepatogastroenterology*, 2012, **59** : 252-254.
3. YU S.C., YEUNG D.T., SO N.M. Imaging features of hepatocellular carcinoma. *Clin. Radiol*., 2004, **59** : 145-156.
4. THOMAS M.B., ABBRUZZESE J.L. Opportunities for targeted therapies in hepatocellular carcinoma. *J. Clin. Oncol*., 2005, **23** : 8093-8108.
5. WOLF P. Thenature and significance of platelet products in human plasma. *Br. J. Haematol*., 1967, **13** : 269-288.
6. MURALIDHARAN-CHARI V., CLANCY J.W., SEDGWICK A., D'SOUZA-SCHOREY C. Microvesicles : mediators of extracellular communication during cancer progression. *J. Cell. Sci*., 2010, **123** : 1603-1611.
7. AL-NEDAWI K., MEEHAN B., MICALLEF J., LHOTAK V., MAY L., GUHA A. *et al*. Intercellular transfer of the oncogenic receptor egfrviii by microvesicles derived from tumour cells. *Nat. Cell. Biol*., 2008, **10** : 619-624.

8. MARTINS V.R., DIAS M.S., HAINAUT P. Tumor-cell-derived microvesicles as carriers of molecular information in cancer. *Curr. Opin. Oncol.*, 2013, **25** : 66-75.
9. SUN W., JULIE LI Y.S., HUANG H.D., SHYY J.Y., CHIEN S. microRNA : a master regulator of cellular processes for bioengineering systems. *Annu. Rev. Biomed. Eng.*, 2010, **12** : 1-27.
10. LU J., GETZ G., MISKA E.A., ALVAREZ-SAAVEDRA E., LAMB J., PECK D. *et al*. MicroRNA expression profiles classify human cancers. *Nature*, 2005, **435** : 834-838.
11. QI J., WANG J., KATAYAMA H., SEN S., LIU S.M. Circulating microRNAs (cmiRNAs) as novel potential biomarkers for hepatocellular carcinoma. *Neoplasma*, 2013, **60** (2) : 135-42.
12. NIEUWLAND R., BERCKMANS R.J., MCGREGOR S., BOING A.N., ROMIJN F.P. *et al*. Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood*, 2000, **95** : 930-935.
13. BRACONI C., HENRY J.C., KOGURE T., SCHMITTGEN T., PATEL T. The role of micrornas in human liver cancers. *Semin. Oncol.*, 2011, **38** : 752-763.
14. BOREL F., KONSTANTINOVA P., JANSEN P.L.M. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. *Journal of Hepatology*, 2012, **56** : 1371-1383.
15. LOFFLER D., BROCKE-HEIDRICH K., PFEIFER G., STOCSITS C., HACKERMULLER J., KRETZSCHMAR A.K. *et al*. Interleukin-6 dependent survivalof multiple myeloma cells involves the Stat3-mediated induction of microRNA-21 through a highly conserved enhancer. *Blood*, 2007, **110** : 1330-1333.
16. GOGA A., BENZ C. Anti-oncomir suppression of tumor phenotypes. *Mol. Interv.*, 2007, **7** : 199-202.
17. YU G.R., KIM S.H., PARK S.H., CUI X.D., XU D.Y., YU H.C. *et al*. Identification of molecular markers for the oncogenic differentiation of hepatocellular carcinoma. *Exp. Mol. Med.*, 2007, **39** : 641-652.
18. CHO S.J., KIM Y.J., SURH Y.J., KIM B.M., LEE S.K. Ibulocydine is a novel prodrug Cdk inhibitor that effectively induces apoptosis in hepatocellular carcinoma cells. *J. Biol. Chem.*, 2011, **286** : 199662-199671.
19. GU Y., LIN S., LI J.L., NAKAGAWA H., CHEN Z., JIN B. *et al*. Altered LKB1/CREB-regulated transcription co-activator (CRTC) signaling axis promotes esophageal cancer cell migration and invasion. *Oncogene*, 2012, **31** : 469-479.
20. STARMANN J., FÄLTH M., SPINDELBÖCK W. *et al*. Gene expression profiling unravels cancer-related hepatic molecular signatures in steatohepatitis but not in steatosis. *PLoS One*, 2012, **7** : e46584.
21. WARD J.J., SODHI J.S., MCGUFFIN L.J., BUXTON B.F., JONES D.T. Prediction and Functional Analysis of Native Disorder in Proteins from the Three Kingdoms of Life. *J. Mol. Biol.*, 2004, **337** : 635-645.
22. PATIL M.A., CHUA M.S., PAN K.H., LIN R., LIH C.J., CHEUNG S.T. *et al*. An integrated data analysis approach to characterize genes highly expressed in hepatocellular carcinoma. *Oncogene*, 2005, **24** : 3737-3747.
23. TOVAR V., ALSINET C., VILLANUEVA A., HOSHIDA Y., CHIANG D.Y., SOLÉ M. *et al*. IGF activation in a molecular subclass of hepatocellular carcinoma and pre-clinical efficacy of IGF-1R blockage. J Hepatol., 2010,52 : 550-559.
24. XIONG W., SUN L.P., CHEN X.M., LI H.Y., JIE S.H. Comparison of microRNA expression profile in HCC-derived microvesicles and the parental cells and evaluation of their roles in HCC. *J. Huazhong U SCI-MED.*, 2013, **33** : 645-648.
25. XU J., ZHU X., WU L., YANG R., YANG Z., WANG Q. *et al*. MicroRNA-122 suppresses cell proliferation and induces cell apoptosis in hepatocellular carcinoma by directly targeting Wnt/β-catenin pathway. *Liver Int.*, 2012, **32** : 752-760.
26. CALVISI D.F., LADU S., GORDEN A., FARINA M., CONNER E.A., LEE J.S. *et al*. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. *Gastroenterology*, 2006, **130** : 1117-1228.