The role of microRNAs in gallbladder cancer (Review)

GANGHUA YANG, LEI ZHANG, RUIXIANG LI and LIN WANG

Department of Geriatric Surgery, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710061, P.R. China

Received October 2, 2015; Accepted March 21, 2016

DOI: 10.3892/mco.2016.905

Abstract. MicroRNAs (also referred to as miRNAs or miRs) play a crucial role in post-transcriptional gene regulation and serve as negative gene regulators by controlling a variety of target genes and regulating diverse biological processes, such as cell proliferation, invasion, migration and apoptosis. Aberrant expression of miRNAs is associated with the development and progression of cancer. Recent studies have reported that miRNAs may repress or promote the expression of cancer-related genes via several different signaling pathways in gallbladder cancer (GBC) patients and may function as tumor suppressors or oncogenes, thus providing a promising tool for the diagnosis and therapeutics of GBCs. In this review, we summarize the role of dysregulawted miRNA expression in the signaling pathways implicated in GBC and discuss the significant role of circulating miRNAs in GBC. Therefore, miRNAs may serve as novel therapeutic targets as well as diagnostic or prognostic markers in GBC.

Contents

1. Introduction

Gallbladder cancer (GBC) is the fifth most common gastrointestinal malignant neoplasm, representing 80-95% of biliary tract cancers, as well as the leading cause of biliary tract malignancy-related mortality worldwide (1,2). Approximately 10,650 cases of GBC and other biliary cancers were diagnosed in the United States in 2014 (3). Despite advances in the treatment of GBC in recent years, the majority of patients eventually develop local recurrent or distant metastatic disease, which is associated with a poor prognosis and an overall 5-year survival rate of <10% (4-7). Early diagnosis, which is crucial for long-term survival of GBC patients, unfortunately occurs only accidentally in patients undergoing cholecystectomy for gallstones or cholecystitis, while the majority of the patients present with advanced metastatic disease due to the absence of specific symptoms and efficient biomarkers (8-12). Additionally, coadjuvant therapy consisting of chemotherapy and/or radiotherapy has not yet proved beneficial in terms of patient survival (13-16). Future research should be focused on developing more effective biomarkers for early diagnosis, therapeutic strategies and prognosis. MicroRNAs (also referred to as miRNAs or miRs) have been widely reported to play a crucial role in the development, metastasis and prognosis of various types of cancer (17-20). In particular, the expression levels of circulating miRNAs differ significantly between cancer patients and healthy volunteers (21-26), which may provide a non-invasive method for early detection.

MicroRNAs are single-stranded RNA molecules that constitute a class of small (~18-25 nucleotides) non-coding RNAs that negatively regulate target genes through transcript degradation and translational inhibition by binding to the 3'-untranslated regions (3'-UTRs) of the target messenger RNAs (27-29). Since their initial discovery in 1993, hundreds of similar miRNAs have been discovered in various types of species (30-32). Accumulating evidence indicates that several diseases, such as cardiovascular, liver, kidney and neurodegenerative diseases, as well as cancer, are initiated or sustained by miRNA dysregulation (28,33-37). Of note, recent studies have demonstrated that miRNAs play vital roles in modulating cell proliferation, invasion, migration and apoptosis. Additionally, miRNAs that act as tumor suppressors and oncogenes have been identified in several cancers (38-41). These exciting results have also been reported in GBC, since the potential association between various miRNAs and GBC was first reported in 2010 (42). Previous studies have established that aberrant expression of miRNAs exerts a significant effect on cancer-related processes by targeting specific genetic alterations, which provide effective biomarkers for diagnosis, therapeutics and prognosis of GBC (43-45). In this study, we describe miRNAs in GBC, particularly their roles as oncogenes and tumor suppressors, their value in diagnosis and prognosis and their potential in providing novel therapeutic strategies for disease management.

Correspondence to: Dr Lin Wang, Department of Geriatric Surgery, First Affiliated Hospital of Xi'an Jiaotong University, 277 West Yanta Road, Xi'an, Shaanxi 710061, P.R. China
E-mail: jiaodawanglin@163.com

Key words: gallbladder cancer, microRNA, biomarker, therapeutic target

2. MicroRNAs and GBCs

miRNA expression profiling has been performed by large-scale microarray analysis in GBC tissues or cells in several studies (26,46-48). Numerous miRNAs exhibit expression changes, with some miRNAs being upregulated, but most being downregulated in GBC cells and tissues. Subsequently, further studies confirmed the function of these miRNAs as either oncogenes or tumor suppressors. In addition, the underlying target genes or mechanisms of miRNA-mediated development and progression of GBC have also been proposed. Furthermore, recent research has demonstrated that circulating miRNA levels were significantly different between GBC patients and healthy volunteers, and were associated with tumor pathological characteristics (26), potentially providing a feasible method for early diagnosis of GBC. A better understanding of the underlying molecular mechanisms of miRNAs may present valuable diagnostic and therapeutic strategies for GBCs (Table I).

3. Oncogenic microRNAs

miR-21. Since the aberrant expression of miR-21 was first reported in human glioblastoma by miRNA profiling (49), there have been several studies indicating that miR-21 exhibited an oncogenic capacity and played an important role in carcinogenesis and progression of various types of cancer (50-52). miR-21 was one of the first aberrant miRNAs identified in GBC by miRNA expression profiling of BK5.erbB2 transgenic mice (53). In this study, 9 miRNAs were found to be significantly upregulated and 13 miRNAs were found to be significantly downregulated in transgenic GBC tissue compared with wild-type tissue. Treatment with the histone deacetylase inhibitor, PCI-24781, significantly decreased the expression of miR-21, as well as miR-142-3p, miR-142-5p and miR-223, which were all upregulated in GBC. In addition, PCI-24781 also induced a significant upregulation in the expression of miR-122, which was downregulated in GBC, highlighting the potential therapeutic value of reversing aberrant miRNA expression by histone deacetylase inhibition in GBCs. Furthermore, research by Sekine *et al* established that miR-21 displayed oncogenic abilities that repress the phosphatase and tensin homolog and may contribute to the role of aquaporins, which are involved in the proliferation, migration, invasion and prognosis of GBC patients (54). Research on targeted therapies against miR-21 may prove to be promising in the suppression of GBC development.

miR-20a. miR-20a, a member of the oncogenic miR-17-92 cluster, was shown to behave as an oncogene by directly or indirectly regulating several target genes or signaling pathways in different types of tumors (55-59). It was previously established that miR-20a plays a central role in the pathogenesis and poor survival of GBC by targeting the mothers against decapentaplegic homolog 7 (Smad7)/β-catenin axis (60). The elevated expression of miR-20a was closely correlated with local invasion, distant metastasis and poor prognosis of GBC patients. Additionally, the aberrant expression of miR-20a induced epithelial-to-mesenchymal transition and enhanced the metastatic potential of GBC cells *in vitro* and *in vivo*. Downregulation of miR-20a by a specific antagomir effectively restored the expression of Smad7 and weakened transforming growth factor (TGF)-β-induced cell metastasis, which may provide a novel therapeutic strategy for GBC patients.

miR-155. Elevated expression of miR-155 has been described in multiple cancers, reflecting tumor staging, progression and treatment outcomes (61-64). In accordance with expectations, miR-155 acts as a vital oncogene in GBCs, as was reported by Kono *et al* (43). Although miR-155 was not upregulated in GBCs compared with pancreaticobiliary maljunction, the expression level of miR-155 was significantly higher in GBCs compared with normal gallbladders. In addition, the overexpression of miR-155 in GBCs was significantly associated with lymph node metastasis and vascular invasion. More importantly, the disease-specific survival rate was significantly lower in GBC patients with high miR-155 expression, compared with that in those with low miR-155 expression. In addition, ectopic expression of miR-155 by transfection significantly enhanced the proliferation and invasion of GBC cells *in vitro*. This provides strong proof that miR-155 may be a useful prognostic factor or tumor marker for therapeutic targeting. However, further research is required in order to identify the underlying mechanisms and target genes downstream of miR-155 in the development and progression of GBC.

miR-182. miR-182 has been reported to significantly regulate cancer progression. Increased expression of miR-182 was associated with poor survival in several types of cancer (65-68). In a study by Qiu *et al*, the expression level of miR-182 was significantly upregulated in GBC compared with that in normal control tissues. Additionally, the expression of miR-182 was significantly higher in gallbladder tumors that eventually metastasized, when compared with primary non-metastatic tumors. Specifically, TGF-β-induced overexpression of miR-182 promoted the migration and invasion of GBC cells, whereas tumor progression was eliminated by miR-182 inhibition (69). In addition, the incidence of pulmonary metastases was inhibited by downregulating the expression of miR-182 using a specific inhibitor *in vivo*. More importantly, the study identified cell adhesion molecule 1 (CADM1) as a novel target gene of miR-182 *in vitro* and *in vivo*, and demonstrated that the ectopic expression of CADM1 in GBC cells partially abrogates miR-182-induced cell invasion.

4. Tumor suppressor microRNAs

miR-218-5p. miR-218-5p was shown to be downregulated in a variety of carcinomas, including cervical, prostate, bladder, pancreatic and esophageal carcinoma, and to exert tumor-suppressive effects (70-74). Previous research has confirmed the marked downregulation of miR-218-5p in GBC compared with paired adjacent normal gallbladder tissue. In addition, miR-218-5p was shown to inhibit GBC cell invasion, migration and proliferation by targeting the polycomb group gene, B-cell-specific moloney murine leukemia virus integration site 1 (Bmi1), with the effects being abrogated by miR-218-5p inhibition (75). Further research revealed that the oncogenic activity of colon cancer-associated transcript-1, a long non-coding RNA (lncRNA), is in part through negative

Table I. The functions of microRNAs in gallbladder cancer.

miRNA (Refs.)	Expression	Sample	Target	Role	Functions
miR-21 (53)	Up	Tissue and cell	PTEN	Oncogene	Proliferation; migration; invasion; apoptosis; prognosis
miR-20a (60)	Up	Tissue and cell	Smad7	Oncogene	Invasion; metastasis; prognosis
miR-155 (43)	Up	Tissue and cell		Oncogene	Invasion; proliferation; lymph node metastasis; prognosis
miR-182 (69)	Up	Tissue and cell	CADM1	Oncogene	Migration; invasion; metastasis
miR-218-5p (75)	Down	Tissue and cell	Bmi1	Tumor suppressor	Invasion; migration; proliferation
miR-335 (44)	Down	Tissue		Tumor suppressor	Histological grade; tumor invasion; lymph node metastasis; pTNM stage; prognosis
miR-34a (82)	Down	Tissue and cell	PNUTS	Tumor suppressor	Proliferation; colony formation; prognosis
miR-130a (86)	Down	Tissue and cell	HOTAIR	Tumor suppressor	Invasion; proliferation
miR-135a-5p (47)	Down	Tissue and cell	VLDLR	Tumor suppressor	pTNM stage; proliferation; colony formation
miR-26a (46)	Down	Tissue and cell	HMGA2	Tumor suppressor	pTNM stage; proliferation
miR-146b-5p (96)	Down	Tissue and cell	EGFR	Tumor suppressor	Tumor size; development; proliferation; apoptosis
miR-1 (48)	Down	Tissue and cell	VEGF-A; AXL	Tumor suppressor	Proliferation; apoptosis
miR-145 (48)	Down	Tissue and cell	AXL	Tumor suppressor	Proliferation; apoptosis
miR-143 (26)	Down	Tissue and blood		Tumor suppressor	Lymph node metastasis; pTNM stage
miR-122 (26) miR-187 (26)	Up	Tissue and blood		Oncogene	Lymph node metastasis; pTNM stage

PTEN, phosphatase and tensin homolog; Smad7, mothers against decapentaplegic homolog 7; CADM1, cell adhesion molecule 1; Bmi1, B-cell-specific moloney murine leukemia virus integration site 1; PNUTS, phosphatase nuclear targeting subunit; HOTAIR, HOX transcript antisense RNA; VLDLR, very low-density lipoprotein receptor; HMGA2, high-mobility group AT-hook 2; EGFR, epidermal growth factor receptor; VEGF-A, vascular endothelial growth factor-A; AXL, AXL receptor tyrosine kinase; pTNM, pathological tumor/node/metastasis.

regulation of miR-218-5p and subsequent modulation of Bmi1 in GBC cells *in vitro* and *in vivo*. Although this provides significant evidence that lncRNAs may function by targeting miRNAs, further investigation is required to identify the association between these two types of RNAs.

miR-335. miR-335 serves as a tumor suppressor miRNA, is transcribed from the genomic region on chromosome 7q32.2 (76) and is downregulated in various human digestive malignancies, such as pancreatic carcinoma, hepatocellular carcinoma, colorectal cancer and gastric cancer (77-80). A similar result was also observed in GBC patients. Previous research has verified that miR-335 is also an important tumor suppressor gene and was significantly downregulated in GBC. The expression level of miR-335 was lower in the majority of GBC tissues compared with that in adjacent normal tissues, as measured by reverse transcription-polymerase chain reaction (RT-PCR). In addition, low expression of miR-335 was correlated with poor histological differentiation, advanced pathological tumor invasion, lymph node metastasis and pathological TNM stage. Importantly, the expression level of miR-335 was an independent prognostic factor for the overall survival of GBC patients by multivariate analysis (44). Unfortunately, the target genes involved in miR-335-mediated tumor suppression in GBC remain unknown.

miR-34a. The miR-34 family, including miR-34a, miR-34b and miR-34c, is directly regulated by p53 and has been reported to induce apoptosis and cell cycle arrest and, thus, act as a tumor suppressor in cancer cells (81). As was expected, low expression of miR-34a has also been found to be important in GBC (82). Additionally, previous studies have demonstrated that altered telomere length may contribute to cancer development and progression (83). In a study by Jin *et al*, miR-34a levels and telomere length were evaluated in 77 gallbladder adenocarcinomas and 36 peritumoral tissues by RT-PCR (82). The results revealed significantly lower expression of miR-34a and longer telomere length in GBC tissues, and, more importantly, that low miR-34a expression was associated with poor GBC patient survival. Interestingly, forced overexpression of miR-34a by an adenovirus may weaken the colony-forming abilities of $CD44^+$ $CD133^+$ GBC tumor stem-like cells *in vitro* and inhibit xenograft tumor growth *in vivo*. Additionally, adenovirus-mediated ectopic expression of miR-34a may

downregulate phosphatase nuclear targeting subunit expression and reduce telomere length in xenograft GBC tumor cells, thus identifying an underlying target gene of miR-34a in GBC.

miR-130a. miR-130a has been confirmed to be downregulated in a variety of carcinomas and to exhibit tumor-suppressive activity (84,85). In GBC, it was previously demonstrated that miR-130a was significantly downregulated in cancer tissues compared with adjacent normal tissue. In addition, miR-130a levels were negatively associated with a lncRNA, HOX transcript antisense RNA (HOTAIR), which has been shown to be a poor prognostic factor in several carcinomas, and to be correlated with tumor metastases. Furthermore, loss of HOTAIR has been associated with the inhibition of cancer invasiveness (86). It was previously demonstrated that the expression of HOTAIR was negatively associated with miR-130a in GBC tissues, and that knockdown of HOTAIR may decrease the invasion of GBC cells, a phenotype that may be partially reversed by miR-130a inhibition. In addition, knockdown of HOTAIR *in vitro* reduced the fraction of cancer cells in S-phase, thus suppressing proliferation, while miR-130a inhibition may reverse this effect. These data provide strong evidence of the inverse association between HOTAIR and miR-130a.

miR-135a-5p. It has been confirmed that miR-135a-5p acts as a tumor suppressor, affecting the proliferation of several carcinomas through interactions with various target genes (87-89). In the study of Zhou *et al*, miR-135a-5p was selected for further investigation due to its aberrant expression and tumor-related functions in GBC, based on miRNA chip and Cell Counting Kit-8 assays, respectively. miR-135a-5p levels were significantly downregulated in GBC tissues, and were correlated with the histological grade of the tumors. Additionally, the expression level of miR-135-5p was found to affect GBC cell proliferation. Specifically, the transfection of a miR-135a-5p mimetic may inhibit the proliferative and colony-forming abilities of GBC cells by arresting the cells in the G1/S phase. Lentivirus-mediated overexpression of miR-135a may significantly decrease the proliferation of GBC cells compared with cells infected with lenti-green fluorescent protein (GFP). Additionally, xenografts established in nude mice derived from the miR-135a-infected cells were significantly smaller compared with those derived from the GFP-infected cells. These data provide proof that miR-135a-5p may inhibit the proliferation of GBC cells *in vitro* and *in vivo*. Furthermore, it was demonstrated that miR-135a exhibited this function through directly binding the 3'-UTR of very low-density lipoprotein receptor, thus resulting in activation of the p38 mitogen-activated protein kinase pathway (47).

miR-26a. miR-26a, located on chromosome 3p22, a region characterized by high frequent loss of heterozygosity in cancer, is a tumor suppressor (90,91). miRNA chip was used to functionally screen for miRNAs in 4 paired GBC and paracancerous tissues, and miR-26a was found to be significantly downregulated in GBC (46). Further investigation revealed that the expression of miR-26a was correlated with pathological TNM stage, and contributed to inhibition of GBC cell proliferation; however, this effect could be reversed by reintroduction of high-mobility group AT-hook 2 (HMGA2), a gene whose expression was negatively associated with miR-26a levels. Thus, miR-26a-induced changes in GBC cell proliferation were mediated by HMGA2.

miR-146b-5p. miR-146b-5p has been reported to possess critical tumor suppressor properties in recent studies (92-95). The expression level of miR-146b-5p was significantly downregulated in GBC tissue compared with that in adjacent tissues, and was found to be significantly correlated with tumor size and development by Cai *et al* (96). Additionally, increased expression of miR-146b-5p in GBC cells may inhibit cell growth by inducing apoptosis and G1 phase arrest. Furthermore, the results demonstrated that epidermal growth factor receptor (EGFR) mRNA levels and miR-146b-5p levels were negatively correlated. EGFR was a direct target of miR-146b-5p and acted as an essential mediator of the cancer-related functions of miR-146b-5p in GBC. In addition, ectopic expression of EGFR may abrogate the inhibition of proliferation induced by miR-146b-5p. These data indicate that the mechanism of action of miR-146b-5p in GBC involves the regulation of EGFR expression.

miR-1 and miR-145. In a previous study, the expression levels of miR-1 and miR-145 were consistently downregulated in GBC compared with normal gallbladder mucosa, as revealed by microarray hybridization, with similar results also observed in GBC cell lines (48). The ectopic expression of miR-1 and miR-145 by microRNA mimetics significantly reduced growth and promoted apoptosis in NOZ cells. Furthermore, the expression level of VEGF-A and AXL mRNAs were significantly decreased in miR-1-transfected cells compared with control-transfected cells. However, in response to miR-145 transfection, the expression of VEGF-A mRNA was unchanged and AXL mRNA was significantly increased, indicating complicated mechanisms that have not yet been elucidated.

5. Circulating microRNAs in GBC

A recent study discovered that the expression levels of 11 miRNAs were altered at least twofold in GBC tissue compared with neighboring non-cancerous gallbladder tissues, as revealed by miRNA microarray analysis (45). Five of these miRNAs, namely miR-21, miR-370, miR-187, miR-122 and miR-202, were upregulated, while 6 miRNAs, namely let-7a, miR-200b, miR-143, miR-31, miR-335 and miR-551, were downregulated. Using blood samples from 40 GBC patients and healthy volunteers, aberrant expression patterns for 6 of these miRNAs (let-7a, miR-21, miR-187, miR-143, miR-202 and miR-335) were confirmed and found to be in agreement with those measured by microarray; in addiiton, the total levels of circulating miRNAs in GBC patients were significantly different compared with those in healthy individuals. Of note, further investigation demonstrated that 3 of the miRNAs (miR-187, miR-143 and miR-122), were correlated with lymph node metastasis and pathological TNM stage. In addition, the association between genetic variants of miRNAs and susceptibility to GBC was also analyzed with blood samples; however, the results demonstrated that common miRNA variants did

not contribute to GBC susceptibility (42). Furthermore, it was demonstrated that the combination of miR-27a$_{rs895819}$, miR-570$_{rs4143815}$ and miR-181a$_{rs12537}$ was the most suitable gene-gene interaction model for predicting susceptibility to GBC and treatment response of GBC patients. Additionally, the interaction of miR-27a$_{rs895819}$ and miR-181a$_{rs12537}$ was correlated with hematological toxicity (neutropenia) in GBC patients undergoing chemoradiotherapy. However, the genetic variants of miRNAs did not affect the response to chemoradiotherapy or the survival outcomes of GBC patients (97). Further research is urgently required to elucidate the important role of circulating miRNAs in GBC.

6. Conclusions

GBC, a common malignant gastrointestinal neoplasm, represents the leading cause of biliary tract malignancy-related mortality, and is currently associated with a significantly lower survival rate compared with a number of other common cancers. MicroRNAs have been widely reported to play crucial roles in cancer development, metastasis and prognosis of GBC patients. In particular, the expression levels of circulating miRNAs differ significantly between cancer patients and healthy volunteers. Elucidating the role of miRNAs in the biology of GBC may provide novel therapeutic strategies for the management of GBC, and identify effective biomarkers for early diagnosis. In this review, we discussed how aberrant miRNA expression has been shown to contribute to the development and progression of GBC, through the upregulation of oncogenic miRNAs and downregulation of tumor-suppressing miRNAs. Additionally, detection of circulating miRNAs may be a non-invasive method, valuable for early diagnosis and prediction of outcome. Importantly, novel, less toxic, miRNA and anti-miRNA therapy has the potential to target multiple genes simultaneously, which provides new tools for the research and development of treatments for GBC.

References

1. Hundal R and Shaffer EA: Gallbladder cancer: Epidemiology and outcome. Clin Epidemiol 6: 99-109, 2014.
2. Lazcano-Ponce EC, Miquel JF, Muñoz N, Herrero R, Ferrecio C, Wistuba II, Alonso de Ruiz P, Aristi Urista G and Nervi F: Epidemiology and molecular pathology of gallbladder cancer. CA Cancer J Clin 51: 349-364, 2001.
3. Siegel R, Ma J, Zou Z and Jemal A: Cancer statistics, 2014. CA Cancer J Clin 64: 9-29, 2014.
4. Ding Q, Li M, Wu X, Zhang L, Wu W, Ding Q, Weng H, Wang X and Liu Y: CLIC1 overexpression is associated with poor prognosis in gallbladder cancer. Tumour Biol 36: 193-198, 2015.
5. Chen L, Wang J, Fu L, Zhang B, Zhang H and Ye B: Prognostic significance of metastasis associated in colon cancer 1 (MACC1) expression in patients with gallbladder cancer. J Cancer Res Ther 10: 1052-1056, 2014.
6. Nigam J, Chandra A, Kazmi HR, Parmar D, Singh D and Gupta V: Prognostic significance of survivin in resected gallbladder cancer. J Surg Res 194: 57-62, 2015.
7. Groot Koerkamp B and Fong Y: Outcomes in biliary malignancy. J Surg Oncol 110: 585-591, 2014.
8. Wang J, Narang AK, Sugar EA, Luber B, Rosati LM, Hsu CC, Fuller CD, Pawlik TM, Miller RC, Czito BG, *et al*: Evaluation of adjuvant radiation therapy for resected gallbladder carcinoma: A multi-institutional experience. Ann Surg Oncol, 2015.
9. Weatherall TJ, Fenton M, Munene G, Dickson PV and Deneve JL: Locally advanced, unresectable squamous cell carcinoma of the gallbladder. Case Rep Surg 2015: 424650, 2015.
10. Yang XW, Yuan JM, Chen JY, Yang J, Gao QG, Yan XZ, Zhang BH, Feng S and Wu MC: The prognostic importance of jaundice in surgical resection with curative intent for gallbladder cancer. BMC Cancer 14: 652, 2014.
11. Niu GC, Shen CM, Cui W and Li Q: Surgical treatment of advanced gallbladder cancer. Am J Clin Oncol 38: 5-10, 2015.
12. Higuchi R, Ota T, Araida T, Kajiyama H, Yazawa T, Furukawa T, Yoshikawa T, Takasaki K and Yamamoto M: Surgical approaches to advanced gallbladder cancer: A 40-year single-institution study of prognostic factors and resectability. Ann Surg Oncol 21: 4308-4316, 2014.
13. Park JO, Oh DY, Hsu C, Chen JS, Chen LT, Orlando M, Kim JS and Lim HY: Gemcitabine plus cisplatin for advanced biliary tract cancer: A systematic review. Cancer Res Treat 47: 343-361, 2015.
14. Alberts SR, Al-Khatib H, Mahoney MR, Burgart L, Cera PJ, Flynn PJ, Finch TR, Levitt R, Windschitl HE, Knost JA and Tschetter LK: Gemcitabine, 5-fluorouracil and leucovorin in advanced biliary tract and gallbladder carcinoma: A North Central Cancer Treatment Group phase II trial. Cancer 103: 111-118, 2005.
15. Ueno H, Okusaka T, Ikeda M, Takezako Y and Morizane C: Phase II study of S-1 in patients with advanced biliary tract cancer. Br J Cancer 91: 1769-1774, 2004.
16. Saito H, Takada T, Miyazaki M, Miyakawa S, Tsukada K, Nagino M, Kondo S, Furuse J, Tsuyuguchi T, Kimura F, *et al*: Radiation therapy and photodynamic therapy for biliary tract and ampullary carcinomas. J Hepatobiliary Pancreat Surg 15: 63-68, 2008.
17. Ohtsuka M, Ling H, Doki Y, Mori M and Calin GA: MicroRNA processing and human cancer. J Clin Med 4: 1651-1667, 2015.
18. Wang J, Du Y, Liu X, Cho WC and Yang Y: MicroRNAs as regulator of signaling networks in metastatic colon cancer. Biomed Res Int 2015: 823620, 2015.
19. Orellana EA and Kasinski AL: MicroRNAs in cancer: A historical perspective on the path from discovery to therapy. Cancers (Basel) 7: 1388-1405, 2015.
20. Bertoli G, Cava C and Castiglioni I: MicroRNAs: New biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer. Theranostics 5: 1122-1143, 2015.
21. Kishikawa T, Otsuka M, Ohno M, Yoshikawa T, Takata A and Koike K: Circulating RNAs as new biomarkers for detecting pancreatic cancer. World J Gastroenterol 21: 8527-8540, 2015.
22. Kelly BD, Miller N, Sweeney KJ, Durkan GC, Rogers E, Walsh K and Kerin MJ: A circulating microRNA signature as a biomarker for prostate cancer in a high-risk group. J Clin Med 4: 1369-1379, 2015.
23. Jia W, Wu Y, Zhang Q, Gao GE, Zhang C and Xiang Y: Expression profile of circulating microRNAs as a promising fingerprint for cervical cancer diagnosis and monitoring. Mol Clin Oncol 3: 851-858, 2015.
24. Karimi Kurdistani Z, Saberi S, Tsai KW and Mohammadi M: MicroRNA-21: Mechanisms of oncogenesis and its application in diagnosis and prognosis of gastric cancer. Arch Iran Med 18: 524-536, 2015.
25. Fang Z, Tang J, Bai Y, Lin H, You H, Jin H, Lin L, You P, Li J, Dai Z, *et al*: Plasma levels of microRNA-24, microRNA-320a and microRNA-423-5p are potential biomarkers for colorectal carcinoma. J Exp Clin Cancer Res 34: 86, 2015.
26. Li G and Pu Y: MicroRNA signatures in total peripheral blood of gallbladder cancer patients. Tumour Biol 36: 6985-6990, 2015.
27. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, *et al*: MicroRNA expression profiles classify human cancers. Nature 435: 834-838, 2005.
28. Di Leva G and Croce CM: miRNA profiling of cancer. Curr Opin Genet Dev 23: 3-11, 2013.
29. Lee RC, Feinbaum RL and Ambros V: The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75: 843-854, 1993.
30. Peterson SM, Thompson JA, Ufkin ML, Sathyanarayana P, Liaw L and Congdon CB: Common features of microRNA target prediction tools. Front Genet 5: 23, 2014.
31. Galli V, Guzman F, de Oliveira LF, Loss-Morais G, Körbes AP, Silva SD, Margis-Pinheiro MM and Margis R: Identifying microRNAs and transcript targets in Jatropha seeds. PLoS One 9: e83727, 2014.
32. Liu B, Li J and Cairns MJ: Identifying miRNAs, targets and functions. Brief Bioinform 15: 1-19, 2014.
33. Shi Q and Yang X: Circulating microRNA and long noncoding RNA as biomarkers of cardiovascular diseases. J Cell Physiol, 2015.

34. Ha TY: MicroRNAs in human diseases: From lung, liver and kidney diseases to infectious disease, sickle cell disease and endometrium disease. Immune Netw 11: 309-323, 2011.
35. Srivastava SP, Koya D and Kanasaki K: MicroRNAs in kidney fibrosis and diabetic nephropathy: Roles on EMT and EndMT. Biomed Res Int 2013: 125469, 2013.
36. Bekris LM and Leverenz JB: The biomarker and therapeutic potential of miRNA in Alzheimer's disease. Neurodegener Dis Manag 5: 61-74, 2015.
37. Shamsuzzama, Kumar L, Haque R and Nazir A: Role of microRNA Let-7 in modulating multifactorial aspect of neurodegenerative diseases: An overview. Mol Neurobiol, 2015 (Epub ahead of print).
38. Xue J, Chen Z, Gu X, Zhang Y and Zhang W: MicroRNA-148a inhibits migration of breast cancer cells by targeting MMP-13. Tumour Biol, 2015 (Epub ahead of print).
39. Tong Z, Liu N, Lin L, Guo X, Yang D and Zhang Q: miR-125a-5p inhibits cell proliferation and induces apoptosis in colon cancer via targeting BCL2, BCL2L12 and MCL1. Biomed Pharmacother 75: 129-136, 2015.
40. Li W, Zhang J, Chen T, Yin P, Yang J and Cao Y: MiR-132 upregulation promotes gastric cancer cell growth through suppression of FoxO1 translation. Tumour Biol, 2015.
41. Farra R, Grassi M, Grassi G and Dapas B: Therapeutic potential of small interfering RNAs/micro interfering RNA in hepatocellular carcinoma. World J Gastroenterol 21: 8994-9001, 2015.
42. Srivastava K, Srivastava A and Mittal B: Common genetic variants in pre-microRNAs and risk of gallbladder cancer in North Indian population. J Hum Genet 55: 495-499, 2010.
43. Kono H, Nakamura M, Ohtsuka T, Nagayoshi Y, Mori Y, Takahata S, Aishima S and Tanaka M: High expression of microRNA-155 is associated with the aggressive malignant behavior of gallbladder carcinoma. Oncol Rep 30: 17-24, 2013.
44. Peng HH, Zhang YD, Gong LS, Liu WD and Zhang Y: Increased expression of microRNA-335 predicts a favorable prognosis in primary gallbladder carcinoma. Onco Targets Ther 6: 1625-1630, 2013.
45. Li Z, Yu X, Shen J, Law PT, Chan MT and Wu WK: MicroRNA expression and its implications for diagnosis and therapy of gallbladder cancer. Oncotarget 6: 13914-13921, 2015.
46. Zhou H, Guo W, Zhao Y, Wang Y, Zha R, Ding J, Liang L, Hu J, Shen H, Chen Z, *et al*: MicroRNA-26a acts as a tumor suppressor inhibiting gallbladder cancer cell proliferation by directly targeting HMGA2. Int J Oncol 44: 2050-2058, 2014.
47. Zhou H, Guo W, Zhao Y, Wang Y, Zha R, Ding J, Liang L, Yang G, Chen Z, Ma B and Yin B: MicroRNA-135a acts as a putative tumor suppressor by directly targeting very low density lipoprotein receptor in human gallbladder cancer. Cancer Sci 105: 956-965, 2014.
48. Letelier P, García P, Leal P, Álvarez H, Ili C, López J, Castillo J, Brebi P and Roa JC: miR-1 and miR-145 act as tumor suppressor microRNAs in gallbladder cancer. Int J Clin Exp Pathol 7: 1849-1867. eCollection 2014, 2014.
49. Chan JA, Krichevsky AM and Kosik KS: MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 65: 6029-6033, 2005.
50. Lin PL, Wu DW, Huang CC, He TY, Chou MC, Sheu GT and Lee H: MicroRNA-21 promotes tumour malignancy via increased nuclear translocation of β-catenin and predicts poor outcome in APC-mutated but not in APC-wild-type colorectal cancer. Carcinogenesis 35: 2175-2182, 2014.
51. Guo Q, Zhang H, Zhang L, He Y, Weng S, Dong Z, Wang J, Zhang P and Nao R: MicroRNA-21 regulates non-small cell lung cancer cell proliferation by affecting cell apoptosis via COX-19. Int J Clin Exp Med 8: 8835-8841. eCollection 2015, 2015.
52. Lu L, Byrnes K, Han C, Wang Y and Wu T: miR-21 targets 15-PGDH and promotes cholangiocarcinoma growth. Mol Cancer Res 12: 890-900, 2014.
53. Kitamura T, Connolly K, Ruffino L, Ajiki T, Lueckgen A, DiGiovanni J and Kiguchi K: The therapeutic effect of histone deacetylase inhibitor PCI-24781 on gallbladder carcinoma in BK5.erbB2 mice. J Hepatol 57: 84-91, 2012.
54. Sekine S, Shimada Y, Nagata T, Sawada S, Yoshioka I, Matsui K, Moriyama M, Omura T, Osawa S, Shibuya K, *et al*: Role of aquaporin-5 in gallbladder carcinoma. Eur Surg Res 51: 108-117, 2013.
55. Zhao S, Yao D, Chen J and Ding N: Circulating miRNA-20a and miRNA-203 for screening lymph node metastasis in early stage cervical cancer. Genet Test Mol Biomarkers 17: 631-636, 2013.
56. Mitani Y, Roberts DB, Fatani H, Weber RS, Kies MS, Lippman SM and El-Naggar AK: MicroRNA profiling of salivary adenoid cystic carcinoma: Association of miR-17-92 upregulation with poor outcome. PLoS One 8: e66778, 2013.
57. Wu Q, Yang Z, Wang F, Hu S, Yang L, Shi Y and Fan D: miR-19b/20a/92a regulates the self-renewal and proliferation of gastric cancer stem cells. J Cell Sci 126: 4220-4229, 2013.
58. Xiong Y, Zhang L and Kebebew E: miR-20a is upregulated in anaplastic thyroid cancer and targets LIMK1. PLoS One 9: e96103, 2014.
59. Qiang XF, Zhang ZW, Liu Q, Sun N, Pan LL, Shen J, Li T, Yun C, Li H and Shi LH: miR-20a promotes prostate cancer invasion and migration through targeting ABL2. J Cell Biochem 115: 1269-1276, 2014.
60. Chang Y, Liu C, Yang J, Liu G, Feng F, Tang J, Hu L, Li L, Jiang F, Chen C, *et al*: miR-20a triggers metastasis of gallbladder carcinoma. J Hepatol 59: 518-527, 2013.
61. Osaka E, Kelly AD, Spentzos D, Choy E, Yang X, Shen JK, Yang P, Mankin HJ, Hornicek FJ and Duan Z: MicroRNA-155 expression is independently predictive of outcome in chordoma. Oncotarget 6: 9125-9139, 2015.
62. Peng Y, Dong W, Lin TX, Zhong GZ, Liao B, Wang B, Gu P, Huang L, Xie Y, Lu FD, *et al*: MicroRNA-155 promotes bladder cancer growth by repressing the tumor suppressor DMTF1. Oncotarget 6: 16043-16058, 2015.
63. Wang P, Zhu CF, Ma MZ, Chen G, Song M, Zeng ZL, Lu WH, Yang J, Wen S, Chiao PJ, *et al*: Micro-RNA-155 is induced by K-Ras oncogenic signal and promotes ROS stress in pancreatic cancer. Oncotarget 6: 21148-21158, 2015.
64. Gasparini P, Cascione L, Fassan M, Lovat F, Guler G, Balci S, Irkkan C, Morrison C, Croce CM, Shapiro CL and Huebner K: MicroRNA expression profiling identifies a four microRNA signature as a novel diagnostic and prognostic biomarker in triple negative breast cancers. Oncotarget 5: 1174-1184, 2014.
65. Wang PY, Gong HT, Li BF, Lv CL, Wang HT, Zhou HH, Li XX, Xie SY and Jiang BF: Higher expression of circulating miR-182 as a novel biomarker for breast cancer. Oncol Lett 6: 1681-1686, 2013.
66. Du C, Weng X, Hu W, Lv Z, Xiao H, Ding C, Gyabaah OA, Xie H, Zhou L, Wu J and Zheng S: Hypoxia-inducible miR-182 promotes angiogenesis by targeting RASA1 in hepatocellular carcinoma. J Exp Clin Cancer Res 34: 67, 2015.
67. Jiang L, Mao P, Song L, Wu J, Huang J, Lin C, Yuan J, Qu L, Cheng SY and Li J: miR-182 as a prognostic marker for glioma progression and patient survival. Am J Pathol 177: 29-38, 2010.
68. Liu R, Li J, Teng Z, Zhang Z and Xu Y: Overexpressed microRNA-182 promotes proliferation and invasion in prostate cancer PC-3 cells by down-regulating N-myc downstream regulated gene 1 (NDRG1). PLoS One 8: e68982, 2013.
69. Qiu Y, Luo X, Kan T, Zhang Y, Yu W, Wei Y, Shen N, Yi B and Jiang X: TGF-β upregulates miR-182 expression to promote gallbladder cancer metastasis by targeting CADM1. Mol Biosyst 10: 679-685, 2014.
70. Yu J, Wang Y, Dong R, Huang X, Ding S and Qiu H: Circulating microRNA-218 was reduced in cervical cancer and correlated with tumor invasion. J Cancer Res Clin Oncol 138: 671-674, 2012.
71. Cheng Y, Yang X, Deng X, Zhang X, Li P, Tao J and Lu Q: MicroRNA-218 inhibits bladder cancer cell proliferation, migration and invasion by targeting BMI-1. Tumour Biol 36: 8015-8023, 2015.
72. Han G, Fan M and Zhang X: MicroRNA-218 inhibits prostate cancer cell growth and promotes apoptosis by repressing TPD52 expression. Biochem Biophys Res Commun 456: 804-809, 2015.
73. Liu Z, Xu Y, Long J, Guo K, Ge C and Du R: MicroRNA-218 suppresses the proliferation, invasion and promotes apoptosis of pancreatic cancer cells by targeting HMGB1. Chin J Cancer Res 27: 247-257, 2015.
74. Tian H, Hou L, Xiong YM, Huang JX, She YJ, Bi XB and Song XR: miR-218 suppresses tumor growth and enhances the chemosensitivity of esophageal squamous cell carcinoma to cisplatin. Oncol Rep 33: 981-989, 2015.
75. Ma MZ, Chu BF, Zhang Y, Weng MZ, Qin YY, Gong W and Quan ZW: Long non-coding RNA CCAT1 promotes gallbladder cancer development via negative modulation of miRNA-218-5p. Cell Death Dis 6: e1583, 2015.
76. Zu Y, Ban J, Xia Z, Wang J, Cai Y, Ping W and Sun W: Genetic variation in a miR-335 binding site in BIRC5 alters susceptibility to lung cancer in Chinese Han populations. Biochem Biophys Res Commun 430: 529-534, 2013.

77. Dohi O, Yasui K, Gen Y, Takada H, Endo M, Tsuji K, Konishi C, Yamada N, Mitsuyoshi H, Yagi N, *et al*: Epigenetic silencing of miR-335 and its host gene MEST in hepatocellular carcinoma. Int J Oncol 42: 411-418, 2013.
78. Xu Y, Zhao F, Wang Z, Song Y, Luo Y, Zhang X, Jiang L, Sun Z, Miao Z and Xu H: MicroRNA-335 acts as a metastasis suppressor in gastric cancer by targeting Bcl-w and specificity protein 1. Oncogene 31: 1398-1407, 2012.
79. Sun Z, Zhang Z, Liu Z, Qiu B, Liu K and Dong G: MicroRNA-335 inhibits invasion and metastasis of colorectal cancer by targeting ZEB2. Med Oncol 31: 982, 2014.
80. Gao L, Yang Y, Xu H, Liu R, Li D, Hong H, Qin M and Wang Y: miR-335 functions as a tumor suppressor in pancreatic cancer by targeting OCT4. Tumour Biol 35: 8309-8318, 2014.
81. Li XJ, Ren ZJ and Tang JH: MicroRNA-34a: A potential therapeutic target in human cancer. Cell Death Dis 5: e1327, 2014.
82. Jin K, Xiang Y, Tang J, Wu G, Li J, Xiao H, Li C, Chen Y and Zhao J: miR-34 is associated with poor prognosis of patients with gallbladder cancer through regulating telomere length in tumor stem cells. Tumour Biol 35: 1503-1510, 2014.
83. Basu N, Skinner HG, Litzelman K, Vanderboom R, Baichoo E and Boardman LA: Telomeres and telomere dynamics: Relevance to cancers of the GI tract. Expert Rev Gastroenterol Hepatol 7: 733-748, 2013.
84. Pan Y, Wang R, Zhang F, Chen Y, Lv Q, Long G and Yang K: MicroRNA-130a inhibits cell proliferation, invasion and migration in human breast cancer by targeting the RAB5A. Int J Clin Exp Pathol 8: 384-393. eCollection 2015, 2015.
85. Li B, Huang P, Qiu J, Liao Y, Hong J and Yuan Y: MicroRNA-130a is down-regulated in hepatocellular carcinoma and associates with poor prognosis. Med Oncol 31: 230, 2014.
86. Ma MZ, Li CX, Zhang Y, Weng MZ, Zhang MD, Qin YY, Gong W and Quan ZW: Long non-coding RNA HOTAIR, a c-Myc activated driver of malignancy, negatively regulates miRNA-130a in gallbladder cancer. Mol Cancer 13: 156, 2014.
87. Dang Z, Xu WH, Lu P, Wu N, Liu J, Ruan B, Zhou L, Song WJ and Dou KF: MicroRNA-135a inhibits cell proliferation by targeting Bmi1 in pancreatic ductal adenocarcinoma. Int J Biol Sci 10: 733-745, 2014.
88. Shin JY, Kim YI, Cho SJ, Lee MK, Kook MC, Lee JH, Lee SS, Ashktorab H, Smoot DT, Ryu KW, *et al*: MicroRNA 135a suppresses lymph node metastasis through down-regulation of ROCK1 in early gastric cancer. PLoS One 9: e85205, 2014.
89. Tang W, Jiang Y, Mu X, Xu L, Cheng W and Wang X: miR-135a functions as a tumor suppressor in epithelial ovarian cancer and regulates HOXA10 expression. Cell Signal 26: 1420-1426, 2014.
90. Yang X, Zhang XF, Lu X, Jia HL, Liang L, Dong QZ, Ye QH and Qin LX: MicroRNA-26a suppresses angiogenesis in human hepatocellular carcinoma by targeting hepatocyte growth factor-cMet pathway. Hepatology 59: 1874-1885, 2014.
91. Liu P, Tang H, Chen B, He Z, Deng M, Wu M, Liu X, Yang L, Ye F and Xie X: miR-26a suppresses tumour proliferation and metastasis by targeting metadherin in triple negative breast cancer. Cancer Lett 357: 384-392, 2015.
92. Shen C, Yang H, Liu H, Wang X, Zhang Y and Xu R: Inhibitory effect and mechanisms of microRNA-146b-5p on the proliferation and metastatic potential of Caski human cervical cancer cells. Mol Med Rep 11: 3955-3961, 2015.
93. Lin F, Wang X, Jie Z, Hong X, Li X, Wang M and Yu Y: Inhibitory effects of miR-146b-5p on cell migration and invasion of pancreatic cancer by targeting MMP16. J Huazhong Univ Sci Technolog Med Sci 31: 509-514, 2011.
94. Wu PY, Zhang XD, Zhu J, Guo XY and Wang JF: Low expression of microRNA-146b-5p and microRNA-320d predicts poor outcome of large B-cell lymphoma treated with cyclophosphamide, doxorubicin, vincristine and prednisone. Hum Pathol 45: 1664-1673, 2014.
95. Katakowski M, Zheng X, Jiang F, Rogers T, Szalad A and Chopp M: miR-146b-5p suppresses EGFR expression and reduces in vitro migration and invasion of glioma. Cancer Invest 28: 1024-1030, 2010.
96. Cai J, Xu L, Cai Z, Wang J, Zhou B and Hu H: MicroRNA-146b-5p inhibits the growth of gallbladder carcinoma by targeting epidermal growth factor receptor. Mol Med Rep 12: 1549-1555, 2015.
97. Gupta A, Sharma A, Yadav A, Rastogi N, Agrawal S, Kumar A, Kumar V, Misra S and Mittal B: Evaluation of miR-27a, miR-181a and miR-570 genetic variants with gallbladder cancer susceptibility and treatment outcome in a north indian population. Mol Diagn Ther 19: 317-327, 2015.