MicroRNA polymorphism: A target for diagnosis and prognosis of hepatocellular carcinoma? (Review)

RUIXIA MA[1,2], MAOMAO ZHAO[1,2], XIN ZOU[1,2], JIANYE ZHOU[3] and ZHONGTIAN BAI[1,2]

[1]The First Clinical Medical College, Lanzhou University; [2]The General Surgery Department, The First Hospital of Lanzhou University; [3]Key Laboratory of Stomatology of State Ethnic Affairs Commission, Northwest Minzu University, Lanzhou, Gansu 730000, P.R. China

Received July 16, 2020; Accepted January 27, 2021

DOI: 10.3892/ol.2021.12586

Abstract. Hepatocellular carcinoma (HCC) is a life-threatening cancer of the digestive system, with complex pathogenesis affected by a broad spectrum of genetic and epigenetic factors. Among several factors, microRNAs (miRNAs), which are considered regulators of the post-transcriptional gene expression, play important roles in determining the malignant phenotype of HCC. In recent years, the advances in molecular genetics have resulted in the characterization of complex genetic factors and in the identification of epigenetic mechanisms of diseases. Accumulating data have suggested that miRNA polymorphisms are involved in tumorigenesis and prognosis, suggesting that the miRNAs may serve as a target for HCC with regard to pathogenesis and prognosis. In the present review, a comprehensive and detailed literature search was conducted and the role of miRNA polymorphisms in the pathogenesis and prognosis of HCC is summarized. The data proposed the use of miRNAs as targets for the diagnosis and treatment of HCC.

Contents

Correspondence to: Dr Zhongtian Bai, The General Surgery Department, The First Hospital of Lanzhou University, 1 DongGang West Road, Chengguan, Lanzhou, Gansu 730000, P.R. China
E-mail: baizht07@lzu.edu.cn

Key words: microRNA, polymorphism, diagnosis, prognosis, hepatocellular carcinoma

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors. It ranks sixth in incidence and fourth in mortality among other types of cancer (1). HCC exhibits poor prognosis mainly due to its undetectable symptoms at the early stages of the disease and high risk of intrahepatic and extrahepatic metastasis during progression. Early diagnosis is a major challenge, due to the lack of a diagnostic target/biomarker allowing specific and sensitive detection of HCC prior to the observation of clear clinical symptoms. The most common marker for the clinical diagnosis of liver cancer is α-fetoprotein (AFP). However, only a small proportion of liver cancer cases present with abnormal AFP serum levels at an early stage (10-20%) (2). AFP-based methods are unsuitable for early diagnosis of patients with AFP-negative HCC in the clinic. Therefore, specifically optimized markers are urgently required for targeting AFP-negative HCC cases. In addition to AFP, several markers have been used for the early detection of HCC (summarized in Table I) (3-7). However, no optimal biomarker has yet been identified.

The occurrence and progression of liver cancer is closely associated with the expression levels of microRNAs (miRNAs/miRs), which can be abnormally regulated by epigenetic modifications, such as alterations in DNA methylation, RNA and histone modifications, ultimately leading to malignant cell transformation. miRNAs are short non-coding single-stranded RNAs that regulate gene expression by binding to the target sites of the 3' untranslated region (3'UTR) of mRNAs. Numerous studies have revealed that the single-nucleotide polymorphisms (SNPs) in miRNA processing machinery genes can affect tumor risk, curative efficacy and prognosis by regulating various signaling pathways (8). The specific genetic variants involved in the miRNA machinery genes disrupt miRNA-dependent regulation by affecting the transcription of the primary transcript and pri/pre-miRNA processing to alter gene expression and susceptibility in cancer (9).

The literature search demonstrated 18 distinct miRNAs with polymorphisms that were specifically associated with

HCC (summarized in Tables II and III). The roles of 7 selected miRNA polymorphisms were examined in the pathogenesis and prognosis of HCC. Subsequently, the applications of miRNA polymorphisms as biomarkers for diagnostic and therapeutic targets were explored. Potential improvements in the sensitivity of these markers may include combined multi-target detection.

2. miR-196a2 polymorphism

miR-196a2 rs11614913 can affect the expression of mature miRNAs and target genes, including homeobox (HOX) and annexin A1 (ANXA1). In various physiological and pathological processes, the HOX gene encodes important transcription factors, while the ANXA1 gene is a mediator of apoptosis and an inhibitor of cell proliferation (10).

Effects of miR-196a2 polymorphism on the risk of HCC are associated with viral hepatitis. Two case-control studies suggested that subjects with hepatitis B virus (HBV), who also carried the miR-196a2 rs11614913 C allele and the CC, CT or CC + CT genotypes, were associated with an increased risk of developing HCC (11,12). The male population with the rs11614913 CC genotype was associated with a high risk of HCC (12). A multi-country meta-analysis indicated that miR-196a2 C>T could lower the risk of HBV/HCV-associated HCC, notably in the Chinese population (13). However, an additional study suggested that rs11614913 may mainly affect the mutation of HBV, but not the susceptibility to HCC (14). The miR-196a2 polymorphism rs12304647 has been shown to be associated with the development of chronic hepatitis-associated HCC. A previous study conducted in South Korea indicated that miR-196a2 rs12304647 AA and AC genotypes in patients with chronic hepatitis decreased the risk of chronic hepatitis-associated HCC compared with subjects with the CC genotype (15).

Effects of miR-196a2 polymorphism on the risk of HCC in patients with liver cirrhosis. Previous studies have shown that the Han national cirrhotic patients with miR-196a2 rs11614913 C allele genotypes (CT and CC) may exhibit increased susceptibility to liver cirrhosis-associated HCC by affecting mature miR-196A expression (16). However, no association has been noted between the incidence of rs11614913 polymorphism and the risk of progression to liver cirrhosis or HCC in Malaysian populations (17). The alleles of the rs12304647 AA or AC genotypes lower the risk of developing HCC associated with liver cirrhosis in Korean and Malaysian populations (17).

Risk of HCC is low in the population with the miR-196a2 rs11614913 T allele. The results of multicenter case-control studies and meta-analyses in Asian and Caucasian populations demonstrated that miR-196a2 rs11614913 TT, CT genotypes and the T allele were significantly associated with low risk of HCC (18-20), whereas the CC genotype and the C allele exhibited a higher risk of HCC (21,22).

The published studies regarding the miR-196a2 polymorphism have mainly focused on rs11614913 and the majority of the studies have shown that the T allele is significantly associated with decreased risk of HCC in Asian and Caucasian populations, patients with HBV or cirrhotic, and male patients with HCC. The overall survival rate of patients with HCC with the T allele was higher, while the results of the studies on the rs12304647 polymorphism were consistent, indicating that the A allele of this site was associated with the lowered risk of HCC in patients with HBV or liver cirrhosis. Therefore, the aforementioned polymorphisms are potentially useful for predicting the risk of HCC in different populations and can be used as early diagnostic tools.

Effects of miR-196a2 rs11614913 polymorphisms on the clinical prognosis of patients with HCC. The miR-196a2 rs11614913 polymorphism was associated with the overall survival rate of patients with HCC (23) and with the tumor size (16,24), whereas the overall survival rate of individuals with the TT genotype was higher than that of subjects with the CT+CC genotype (23). The donor miR-196a2 rs11614913 polymorphism was associated with HCC recurrence following liver transplantation by regulating the tumor microenvironment (24).

3. miR-146a polymorphism

miR-146a inhibits the proliferation and metastasis of cancer cells and promotes their apoptosis by blocking the nuclear factor κB pathway (25).

miR-146a rs2910164 G allele may increase the risk of developing HCC. A meta-analysis of a large number of cases in China, Korea and Turkey has shown that individuals with the miR-146a rs2910164 G allele and the GG genotype exhibit a higher risk of developing HBV-associated HCC compared with those with the C allele and the CC genotype (13). The C allele, and the CC or CG genotypes were associated with low risk of HCC in Asians. Sex differences, in the C allele and in the CC genotype decreased the risk for developing HCC, whereas no significant association was noted between SNPs in female subjects and HCC risk (20). The rs2910164 G allele increases the risk of HCC in Asian populations (22,26), notably in the Chinese populations (26). However, certain studies have shown no significant association between the incidence of the miR-146a G>C polymorphism and the risk of HCC (17,18,27,28). In summary, the risk of HCC was significantly increased in Asian patients with HBV with the miR-146a rs2910164 G allele, notably in China. The G allele at this site is expected to be a predictor of HCC risk in the aforementioned specific population.

4. miR-499 polymorphism

miR-499 and miR-1 inhibit the expression levels of Ets1 by binding to the 3'UTR of the mRNA molecule of the proto-oncogene ets1. This process reduces the invasion and migration of HepG2 cells induced by HGF (29). The miR-499 polymorphism has been investigated with regard to rs3746444.

Effects of miR-499 polymorphism on the risk of HCC. The miR-499 rs3746444 C allele caused a significant increase in the risk of HCC compared with that noted for the T allele (22,28). However, previous studies suggested the lack of significant associations between the incidence of the miR-499 T>C polymorphism and the risk of HCC (21,27). A previous study demonstrated that the presence of the A>G allele at the miR-499 rs3746444 site was significantly associated with the risk of developing HCC in a recessive model (20), whereas the

Table I. Summary of HCC biomarkers.

Biomarker	Source	Application of biomarker in HCC	View of clinical utility	(Refs.)
AFP	Serum/plasma	Early diagnosis, monitoring and recurrence	Low sensitivity	(2)
GPC3	Serum/plasma and tissues	Early diagnosis and discriminating tumor cells from benign hepatic cells	Combination of AFP significantly elevate the sensitivity and specificity	(3)
CTCs	Serum/plasma	Diagnostic	The detection of CTCs is technically challenging	(4)
GP73	Serum/plasma	Early diagnosis, and recurrence	High sensitivity and specificity	(5)
AFP-L3%	Serum/plasma	Early diagnosis and screening	The combination of AFP, AFP-L3%, and DCP may be useful in the diagnosis and screening	(6)
OPN	Serum	Diagnosis and screening	High sensitivity and specificity	(7)

HCC, hepatocellular carcinoma; AFP, α-fetoprotein; GPC-3, glypican-3; CTCs, circulating tumors cells; GP73, Golgi protein 73; DCP, des-γ-carboxy prothrombin; OPN, osteopontin.

Table II. Pattern of miRNA polymorphism in HCC.

miRNA	SNP	Allele gene	Susceptibility or prognosis[a]	(Refs.)
miR-196a2	rs11614913	C/T	C(+)	(12,13,16,21,22)
			T(-)	(12,13,18-20,23,24)
			=	(17)
	rs12304647	A/C	A(-)	(15,17)
miR-146a	rs2910164	C/G	G(+)	(13,22,26)
			C(-)	(20)
			=	(17,18,27,28)
miR-499	rs3746444	A/G	G(+)	(20,21,23)
			G(-)	(30)
			=	(18,28)
		C/T	C(+)	(22,28)
			=	(27)
miR-149	rs2292832	T/C	C(-)	(30,33)
			T(+)	(33)
miR-34b/c	rs4938723	C/T	C(+)	(14,36)
			=	(36)
miR-122	rs1135519	C/T	C(+)	(38)
			=	(38)
	rs9966765	C/T	=	(38)
	rs17669	G/A	=	(38)
	rs4309483	C/A	C(+)	(39)
	rs3783553	Ins/Del	Del(+)	(27)
miR-218	rs11134527	G/A	G(+)	(41)
			=	(27)

[a]Susceptibility or prognosis of HCC: (-), allele is associated with decreased susceptibility to HCC or good prognosis; (+), allele is associated with increased susceptibility to HCC or poor prognosis; =, polymorphism is not associated with susceptibility or prognosis of HCC. miRNA/miR, microRNA; HCC, hepatocellular carcinoma; SNP, single-nucleotide polymorphism.

rs3746444 G allele was associated with high risk of non-HBV HCC (21), suggesting that the HBV played an important role in the presence of miR-499 polymorphisms and HCC risk assessment. In addition, the association between the incidence of miR-499 polymorphisms and HCC risk was different in different populations. The rs3746444 AG+GG genotype

exhibited a lower HCC risk compared with the AA genotype in a Korean population (30). This association was not found in the Chinese population (18,28).

Effects of miR-499 polymorphism on the clinical prognosis of patients with HCC. Under different dominant inheritance patterns, the levels of serum bilirubin, globulin, γ-glutamyl transpeptidase, alkaline phosphatase and serum cholinesterase, in patients with HCC who carried the miR-499 rs3746444 C allele, were lower compared with those who carried other alleles (22). Subjects with the TC+CC genotype were more likely to develop advanced HCC with larger tumor size and/or higher total bilirubin levels compared with subjects carrying the TT genotype (31). The overall survival rate of patients with the AA genotype of miR-499a was higher compared with that of the patients with the AG+GG genotype (23).

5. miR-149 polymorphism

miR-149 inhibits the invasion and migration of HCC by directly targeting Mg^{2+}/Mn^{2+}-dependent protein phosphatase 1F (32). The investigation of the miR-149 polymorphism was mainly focused on rs2292832.

Effects of miR-149 polymorphism on the risk of HBV-associated HCC. In the Korean population, patients with HBV and miR-149 rs2292832 CT or CT+CC genotypes exhibited a significantly lower risk of HCC and, therefore, miR-149 may be considered a prognostic biomarker of OKUDA II patients with HCC (30). However, in the Chinese population, HBV patients with miR-149 rs2292832 CT+CC or CT+TT genotypes exhibited a higher risk of HCC (33), indicating that the miR-149 polymorphism exerted different effects on the risk of HBV-associated HCC in different populations. In the Iranian population, the frequency of the CC genotype in miR-149 rs2292832 female patients with HCC was higher compared with that noted in the female control group (11).

6. miR-34 polymorphism

miR-34 is an evolutionarily conserved miRNA family, including the miR-34a, b and c forms. miR-34 is the target of the p53 gene, which encodes transcription factors involved in growth inhibition and apoptotic pathways (34).

miR-34b/c rs4938723C alleles increase the risk of HCC. Previous studies have shown that the incidence of miR-34b/c rs4938723 C alleles in Chinese and Korean populations increases their risk of developing HCC (14,35), notably in women and patients with HBV (14). In addition, the combination of miR-34b/c rs4938723 CC-TP53 rs1042522 Arg/Arg genotypes significantly increased the risk of developing HCC in Korean populations (35). According to two meta-analyses performed in China, Korea and Turkey, the miR-34b/c rs4938723 C allele was associated with increased risk of HCC (27,36). However, a meta-analysis including only Chinese and Korean populations did not result to this outcome (36). Due to the small sample size used in that study, the association between this polymorphism and the risk of HCC may not be representative of the entire population and requires to be determined in a larger sample size. In conclusion, the current research provided evidence to support the association between miR-34b/c rs4938723 C alleles and the increased risk of HCC.

7. miR-122 polymorphism

miR-122 can downregulate the transcriptional activity of β-catenin by decreasing BCL9 expression, which results in the regulation of the WNT/β-catenin signaling pathway and inhibits the growth of HCC (37).

A miR-122 polymorphism is associated with an increased risk of HCC. The miR-122 rs1135519 CC genotypes alone or in combination with the miR-122 rs9966765 CC genotypes can decrease the expression levels of miR-122, resulting in an increased risk of HCC (38). The miR-122 rs4309483 C alleles can also decrease the expression levels of miR-122, which lowers the risk of chronic HBV infection and increases the risk of HCC in HBV carriers (39). A multi-country meta-analysis indicated that the miR-122 rs3783553 Del allele may increase the risk of HCC (27). The exact molecular mechanism by which certain SNPs alters the expression levels of miR-122 requires further investigation in future studies.

8. miR-218 polymorphism

A previous study demonstrated that miR-218 could reverse the epithelial-mesenchymal transformation to the mesenchymal-epithelial transformation by targeting the RNA binding protein SERBP1. This process resulted in the inhibition of cell metastasis (40). The miR-218 rs11134527 AG or GG+AG genotypes significantly increased the risk of HBV-associated HCC (41). The AG genotypes at this site were associated with family history and elevated serum AFP levels (20 ng/ml) (42). However, a previous study demonstrated no significant association between the incidence of the miR-218 A>G polymorphism and the risk of HCC (27).

9. Other miRNA polymorphisms

miRNA polymorphisms affecting the risk of HCC associated with HBV/liver cirrhosis. The miR-106b-25 rs999885 variant genotypes (AG/GG) may contribute to the overexpression of miR-106b-5p, inhibiting cell survival, metastasis and tumor recurrence by decreasing the expression of X-linked inhibitor of apoptosis protein (43). The change in the polymorphic variant rs999885 (A to G) may provide protection against chronic HBV infection. In contrast to these studies, it has also been suggested that the risk of HCC in persistent HBV carriers is increased by alteration in the expression levels of miR-106b-25 clusters (44). Notably, this association was not found in a multi-country meta-analysis (27). The mechanisms associated with the incidence of miR-106b-25 rs999885 may be more complicated.

Previous studies performed in different regions of China indicated that the incidence of the pri-miR-378 rs1076064 G allele was associated with a lower risk of HCC in HBV carriers and that patients with HCC and the G allele exhibited higher survival rate (45). In the Korean population, it was shown that the miR-604 rs2368392 T allele may be a risk allele for chronic HBV infection, whereas it may be a

Table III. Pattern of other miRNA polymorphisms in HCC.

miRNA	SNP	Allele gene	Susceptibility or prognosis[a]	(Refs.)
miR-106	rs999885	A/G	G(-)	(44)
			=	(27)
miR-449b	rs10061133	G/A	G(+)	(48)
miR-3152	rs13299349	G/A	A(+)	(48,49)
miR-let-7c	rs6147150	Ins/Del	Del(+)	(27)
MET	rs1621	G/A	G(-)	(50)
miR-199a	rs74723057	C/G	=	(50)
pre-miR-3131	rs57408770	Ins/Del	Ins(+)	(51)
miR-920	rs16405	Ins/Del	Del(-)	(27)
miR-608	rs4919510	C/G	C(+)	(53)
			=	(30)
miR-502	rs16917496	C/T	C(-)	(57)
miR-492	rs2289030	G/C	G(-)	(54)
miR-155	rs767649	A/T	T(+)	(56)

[a]Susceptibility or prognosis of HCC; (-), allele is associated with decreased susceptibility to HCC or good prognosis; (+), allele is associated with increased susceptibility to HCC or poor prognosis; =, polymorphism is not associated with susceptibility or prognosis of HCC. miRNA/miR, microRNA; HCC, hepatocellular carcinoma; SNP, single-nucleotide polymorphism.

protective allele for the development of HCC in chronic HBV carriers (46). In chronic HBV carriers and patients with liver cirrhosis, the pri-miRNAs-371-373 rs3859501 A allele and the pri-miRNAs-371-373_ht2 (C-A-C) haplotype decreased the incidence of HCC (47).

miRNA polymorphisms are associated with increased risk of HCC. It has been shown that the G allele of miR-449b rs10061133 can increase the susceptibility to HCC (48), whereas the miR-1269a rs73239138 A allele may increase the susceptibility to HCC by lowering the expression levels of miR-1269a in HCC cells or by weakening the binding to its target genes (49). In addition, the miR-3152 rs13299349 AA genotype or the A allele (48) and the miR-let-7c rs6147150 Del mutation (27) may be associated with increased risk of HCC.

miRNA polymorphisms associated with lowered risk of HCC. The association between miR-199a rs74723057 and HCC risk was not observed in the population study conducted in South China. However, the data collected in the population with the miR-199a rs74723057 CG/GG genotype demonstrated that subjects with the MET rs1621 GG genotype indicated a lower risk of HCC compared with those with the AG/AA genotype (50). MET rs1621 polymorphism alone or in combination with miR-199a rs74723057 polymorphism may affect the susceptibility to HCC, as demonstrated in the population of South China (50). A multinational meta-analysis demonstrated that the miR-920 rs16405 Del allele may lower the risk of HCC (27). In the Chinese population, the insertion of the pre-miR-3131 rs5740877 allele may affect the expression of mature miR-3131 and its target genes DTHD1 and XAF1 by altering the recognition of the CNNC recognition sequence by SRp20 and its binding to the precursor of miR-3131. This in turn will promote the proliferation of HCC cells and the inhibition of their apoptosis (51). This locus is likely to become a marker for personalized diagnosis of HCC.

miRNA polymorphisms associated with clinical prognosis in patients with HCC. Previous studies have shown that miR-608 may inhibit the proliferation of HCC cells by targeting the BET family protein BRD4 (52). However, certain studies have shown that the miR-608 rs4919510 polymorphism is not associated with the risk of HCC (33,53). The miR-492 rs2289030 CG and CG+GG genotypes significantly lowered the risk of death in patients with HCC compared with that noted in carriers of the CC genotype (54). This finding may improve treatment decisions in patients with HCC. The miR-1268a rs28599926 T allele may increase the risk of aflatoxin B1 (AFB1)-associated HCC by modifying a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4). This allele was also associated with larger tumor size, higher portal vein tumor risk, tumor dedifferentiation, increased AFB1 adduct levels and high mutation risk of the TP53 gene (55). The polymorphism of miR-1268a may be a biomarker of risk and prognosis of AFB1-associated HCC. The miR-155 rs767649 T allele increases the risk of poor survival and of HCC incidence (56).

In summary, the patients with miR-608 rs4919510 CC, miR-492 rs2289030 CG and CG+GG genotypes exhibited a more favorable disease prognosis, while patients with HCC with miR-1268a rs28599926 T and miR-155 rs767649 T alleles exhibited a poor prognosis.

10. Lack of association of miRNA gene polymorphisms with the risk of HCC

Previous studies have shown that the SET8rs16917496 T/C polymorphism (57) as well as certain polymorphisms in miR-301b (22) and miR-423 (31) are not significantly

associated with the occurrence, development and prognosis of HCC.

11. Conclusion

In summary, the present review indicated that the polymorphic sites of various miRNAs were associated with HCC. Notably, miR-146a rs2910164, miR-196a2 rs11614913 and miR-34b/c rs4938723 polymorphisms played an important role in the occurrence, development and prognosis of HCC, while the conclusions drawn regarding the miR-499 rs3746444 and miR-149 rs2292832 polymorphisms were different in different populations. This is possibly associated with various factors, such as ethnic heterogeneity of the study population, differences in inclusion and exclusion criteria, different sample sizes and different selection of statistical methods. In addition, one miRNA polymorphism may not be sufficient as a marker for the diagnosis or treatment of HCC, whereas a panel of miRNA polymorphisms holds the potential as a new approach to improve the diagnosis and treatment of this disease.

The investigations on the association between miRNA polymorphisms and HCC incidence are mainly concentrated in Asia and the subjects examined are mainly patients with HCC from hospitals and normal individuals without liver disease. The samples are not fully representative of the worldwide population and additional research on multiple regions and different stages of HCC will aid the clarification of the associations between the different parameters. The mechanisms of miRNA involved in the occurrence, development and prognosis of HCC are not fully understood and the interactions among multiple miRNA polymorphisms require additional studies. As the awareness of HCC and miRNA continues to improve, the correlation analysis of larger sample sizes will provide additional insight into the main pathogenic miRNA of HCC and the mechanism of various miRNAs in the future, which will result in improved prevention and treatment of patients with HCC.

Acknowledgements

Not applicable.

Funding

This work was supported by grants of the National Natural Science Fund of China (grant nos. 82060666 and 81702326).

Availability of data and materials

Not applicable.

Authors' contributions

JZ, ZB and RM conceived and designed the review. RM, MZ and XZ wrote the manuscript and prepared the tables. ZB and RM reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. European Association for the Study of the Liver: EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 69: 182–236, 2018.
3. Zhou F, Shang W, Yu X and Tian J: Glypican-3: A promising biomarker for hepatocellular carcinoma diagnosis and treatment. Med Res Rev 38: 741-767, 2018.
4. Harding JJ, Khalil DN and Abou-Alfa GK: Biomarkers: What role do they play (If Any) for diagnosis, prognosis and tumor response prediction for hepatocellular carcinoma? Dig Dis Sci 64: 918-927, 2019.
5. WangY and WanYY: Golgi protein 73, hepatocellular carcinoma and other types of cancers. Liver Res 4: 161-167, 2020.
6. Wang X, Zhang Y, Yang N, He H, Tao X, Kou C and Jiang J: Evaluation of the combined application of AFP, AFP-L3%, and DCP for hepatocellular carcinoma diagnosis: A Meta-analysis. Biomed Res Int 2020: 5087643, 2020.
7. Zhu M, Zheng J, Wu F, Kang B, Liang J, Heskia F, Zhang X and Shan Y: OPN is a promising serological biomarker for hepatocellular carcinoma diagnosis. J Med Virol: Feb 11, 2021 (Epub ahead of print). doi: 10.1002/jmv.25704.
8. Yan Z, Zhou Z, Li C, Yang X, Yang L, Dai S, Zhao J, Ni H, Li S and Yao Y: Polymorphisms in miRNA genes play roles in the initiation and development of cervical cancer. J Cancer 10: 4747-4753, 2019.
9. Ryan BM: microRNAs in cancer susceptibility. Adv Cancer Res 135: 151-171, 2017.
10. Zóia MA, Azevedo FV, Vecchi L, Mota ST, Rodovalho VR, Cordeiro AO, Correia LI, Silva AC, Ávila VM, Araújo TG and Goulart LR: Inhibition of triple-negative breast cancer cell aggressiveness by cathepsin D blockage: Role of Annexin A1. Int J Mol Sci 20: 1337, 2019.
11. Farokhizadeh Z, Dehbidi S, Geramizadeh B, Yaghobi R, Malekhosseini SA, Behmanesh M, Sanati MH, Afshari A, Moravej A and Karimi MH: Association of MicroRNA polymorphisms with hepatocellular carcinoma in an iranian population. Ann Lab Med 39: 58-66, 2019.
12. Akkız H, Bayram S, Bekar A, Akgöllü E and Ulger Y: A functional polymorphism in pre-microRNA-196a-2 contributes to the susceptibility of hepatocellular carcinoma in a Turkish population: A case-control study. J Viral Hepat 18: e399-e407, 2011.
13. Tian T, Wang M, Zhu W, Dai ZM, Lin S, Yang PT, Liu XH, Liu K, Zhu YY, Zheng Y, *et al*: MiR-146a and miR-196a-2 polymorphisms are associated with hepatitis virus-related hepatocellular cancer risk: A meta-analysis. Aging (Albany NY) 9: 381-392, 2017.
14. Han Y, Pu R, Han X, Zhao J, Zhang Y, Zhang Q, Yin J, Xie J, Shen Q, Deng Y, *et al*: Associations of pri-miR-34b/c and pre-miR-196a2 polymorphisms and their multiplicative interactions with hepatitis B virus mutations with hepatocellular carcinoma risk. PLoS One 8: e58564, 2013.
15. Kim HY, Yoon JH, Lee HS, Cheong JY, Cho SW, Shin HD and Kim YJ: MicroRNA-196A-2 polymorphisms and hepatocellular carcinoma in patients with chronic hepatitis B. J Med Virol 86: 446-453, 2014.
16. Li XD, Li ZG, Song XX and Liu CF: A variant in microRNA-196a2 is associated with susceptibility to hepatocellular carcinoma in Chinese patients with cirrhosis. Pathology 42: 669-673, 2010.
17. Riazalhosseini B, Mohamed Z, Apalasamy YD, Eng HS and Mohamed R: Association between microRNA-196A2 and microRNA-146A polymorphisms and progression to cirrhosis and hepatocellular carcinoma in patients with viral hepatitis B. Pharmacogenet Genomics 26: 74-79, 2016.
18. Kou JT, Fan H, Han D, Li L, Li P, Zhu J, Ma J, Zhang ZH and He Q: Association between four common microRNA polymorphisms and the risk of hepatocellular carcinoma and HBV infection. Oncol Lett 8: 1255-1260, 2014.

19. Kang Z, Li Y, He X, Jiu T, Wei J, Tian F and Gu C: Quantitative assessment of the association between miR-196a2 rs11614913 polymorphism and cancer risk: Evidence based on 45,816 subjects. Tumour Biol 35: 6271-6282, 2014.
20. Yu JY, Hu F, Du W, Ma XL and Yuan K: Study of the association between five polymorphisms and risk of hepatocellular carcinoma: A meta-analysis. J Chin Med Assoc 80: 191-203, 2017.
21. Zheng L, Zhuang C, Zhao J and Ming L: Functional miR-146a, miR-149, miR-196a2 and miR-499 polymorphisms and the susceptibility to hepatocellular carcinoma: An updated meta-analysis. Clin Res Hepatol Gastroenterol 41: 664-676, 2017.
22. Qi JH, Wang J, Chen J, Shen F, Huang JT, Sen S, Zhou X and Liu SM: High-resolution melting analysis reveals genetic polymorphisms in microRNAs confer hepatocellular carcinoma risk in Chinese patients. BMC Cancer 14: 643, 2014.
23. Qiu GP and Liu J: MicroRNA gene polymorphisms in evaluating therapeutic efficacy after transcatheter arterial chemoembolization for primary hepatocellular carcinoma. Genet Test Mol Biomarkers 205: 579-586, 2016.
24. Xu X, Ling Q, Wang J, Xie H, Wei X, Lu D, Hu Q, Zhang X, Wu L, Zhou L and Zheng S: Donor miR-196a-2 polymorphism is associated with hepatocellular carcinoma recurrence after liver transplantation in a Han Chinese population. Int J Cancer 138: 620-629, 2016.
25. Bhaumik D, Scott GK, Schokrpur S, Patil CK, Campisi J and Benz CC: Expression of microRNA-146 suppresses NF-kappaB activity with reduction of metastatic potential in breast cancer cells. Oncogene 27: 5643-5647, 2008.
26. Wang Z, Zhang L, Shi X, Xu H, Wang T and Bian J: Association between two common polymorphisms and risk of hepatocellular carcinoma: Evidence from an updated meta-analysis. Biomed Res Int 2014: 468605, 2014.
27. Wang BS, Liu Z, Xu WX and Sun SL: Functional polymorphisms in microRNAs and susceptibility to liver cancer: A meta-analysis and meta-regression. Genet Mol Res 13: 5426-5440, 2014.
28. Hu M, Zhao L, Hu S and Yang J: The association between two common polymorphisms in MicroRNAs and hepatocellular carcinoma risk in Asian population. PLoS One 8: e57012, 2013.
29. Wei W, Hu Z, Fu H, Tie Y, Zhang H, Wu Y and Zheng X: MicroRNA-1 and microRNA-499 downregulate the expression of the ets1 proto-oncogene in HepG2 cells. Oncol Rep 28: 701-706, 2012.
30. Kim WH, Min KT, Jeon YJ, Kwon CI, Ko KH, Park PW, Hong SP, Rim KS, Kwon SW, Hwang SG, *et al*: Association study of microRNA polymorphisms with hepatocellular carcinoma in Korean population. Gene 504: 92-97, 2012.
31. Ma Y, Wang R, Zhang J, Li W, Gao C, Liu J and Wang J: Identification of miR-423 and miR-499 polymorphisms on affecting the risk of hepatocellular carcinoma in a large-scale population. Genet Test Mol Biomarkers 18: 516-524, 2014.
32. Luo G, Chao YL, Tang B, Li BS, Xiao YF, Xie R, Wang SM, Wu YY, Dong H, Liu XD, *et al*: miR-149 represses metastasis of hepatocellular carcinoma by targeting actin-regulatory proteins PPM1F. Oncotarget 6: 37808-37823, 2015.
33. Wang R, Zhang J, Ma Y, Chen L, Guo S, Zhang X, Ma Y, Wu L, Pei X, Liu S, *et al*: Association study of miR-149 rs2292832 and miR-608 rs4919510 and the risk of hepatocellular carcinoma in a large-scale population. Mol Med Rep 10: 2736-2744, 2014.
34. Roy S, Levi E, Majumdar AP and Sarkar FH: Expression of miR-34 is lost in colon cancer which can be re-expressed by a novel agent CDF. J Hematol Oncol 5: 58, 2012.
35. Son MS, Jang MJ, Jeon YJ, Kim WH, Kwon CI, Ko KH, Park PW, Hong SP, Rim KS, Kwon SW, *et al*: Promoter polymorphisms of pri-miR-34b/c are associated with hepatocellular carcinoma. Gene 524: 156-160, 2013.
36. Liu CJ, Ma XW, Zhang XJ and Shen SQ: pri-miR-34b/c rs4938723 polymorphism is associated with hepatocellular carcinoma risk: A case-control study in a Chinese population. Int J Mol Epidemiol Genet 8: 1-7, 2017.
37. Luna JM, Barajas JM, Teng KY, Sun HL, Moore MJ, Rice CM, Darnell RB and Ghoshal K: Argonaute CLIP defines a deregulated miR-122-bound transcriptome that correlates with patient survival in human liver cancer. Mol Cell 67: 400-410.e7, 2017.
38. Bei C, Liu S, Yu X, Qiu M, Tang B, Liao W, He S and Yu H: Single nucleotide polymorphisms in miR-122 are associated with the risk of hepatocellular carcinoma in a southern Chinese population. Biomed Res Int 2018: 1540201, 2018.
39. Liu Y, Xie K, Wen J, Deng M, Li J and Hu Z: A genetic variant in microRNA-122 regulatory region confers risk for chronic hepatitis B virus infection and hepatocellular carcinoma in Han Chinese. J Med Virol 86: 1669-1674, 2014.
40. Wang T, Xu L, Jia R and Wei J: MiR-218 suppresses the metastasis and EMT of HCC cells via targeting SERBP1. Acta Biochim Biophys Sin (Shanghai) 49: 383-391, 2017.
41. Han Y, Pu R, Han X, Zhao J, Li W, Yin J, Zhang Y, Shen Q, Xie J, Zhang Q, *et al*: Association of a potential functional pre-miR-218 polymorphism and its interaction with hepatitis B virus mutations with hepatocellular carcinoma risk. Liver Int 34: 728-736, 2014.
42. Zhang LS, Liang WB, Gao LB, Li HY, Li LJ, Chen PY, Liu Y, Chen TY, Han JG, Wei YG and Zhang L: Association between pri-miR-218 polymorphism and risk of hepatocellular carcinoma in a Han Chinese population. DNA Cell Biol 31: 761-765, 2012.
43. Qi F, Huang M, Pan Y, Liu Y, Liu J, Wen J, Xie K, Shen H, Ma H, Miao Y and Hu Z: A genetic variant in the promoter region of miR-106b-25 cluster predict clinical outcome of HBV-related hepatocellular carcinoma in Chinese. PLoS One 9: e85394, 2014.
44. Liu Y, Zhang Y, Wen J, Liu L, Zhai X, Liu J, Pan S, Chen J, Shen H and Hu Z: A genetic variant in the promoter region of miR-106b-25 cluster and risk of HBV infection and hepatocellular carcinoma. PLoS One 7: e32230, 2012.
45. An J, Liu J, Liu L, Liu Y, Pan Y, Huang M, Qi F, Wen J, Xie K, Ma H, *et al*: A genetic variant in primary miR-378 is associated with risk and prognosis of hepatocellular carcinoma in a Chinese population. PLoS One 9: e93707, 2014.
46. Cheong JY, Shin HD, Cho SW and Kim YJ: Association of polymorphism in microRNA 604 with susceptibility to persistent hepatitis B virus infection and development of hepatocellular carcinoma. J Korean Med Sci 29: 1523-1527, 2014.
47. Kwak MS, Lee DH, Cho Y, Cho EJ, Lee JH, Yu SJ, Yoon JH, Lee HS, Kim CY, Cheong JY, *et al*: Association of polymorphism in pri-microRNAs-371-372-373 with the occurrence of hepatocellular carcinoma in hepatitis B virus infected patients. PLoS One 7: e41983, 2012.
48. Li W, Ma Y, Zeng D, Zhang J, Wang R, Hu J, Yang D, Hu H, Wang J and Liu J: Association between microRNA single nucleotide polymorphisms and the risk of hepatocellular carcinoma. Rev Med Chil 144: 508-515, 2016.
49. Min P, Li W, Zeng D, Ma Y, Xu D, Zheng W, Tang F, Chen J, Shi J, Hu H, *et al*: A single nucleotide variant in microRNA-1269a promotes the occurrence and process of hepatocellular carcinoma by targeting to oncogenes SPATS2L and LRP6. Bull Cancer 104: 311-320, 2017.
50. Wang Q, Yu X, Li Q, Qin L, Tan S, Zeng X, Qiu X, Tang B, Jin J, Liao W, *et al*: Association between miR-199a rs74723057 and MET rs1621 polymorphisms and the risk of hepatocellular carcinoma. Oncotarget 7: 79365-79371, 2016.
51. Wang C, Li L, Yin Z, Zhang Q, Zhao H, Tao R, Wang S, Hu S, He Y, Wang D, *et al*: An indel polymorphism within pre-miR3131 confers risk for hepatocellular carcinoma. Carcinogenesis 38: 168-176, 2017.
52. He L, Meng D, Zhang SH, Zhang Y, Deng Z and Kong LB: microRNA-608 inhibits human hepatocellular carcinoma cell proliferation via targeting the BET family protein BRD4. Biochem Biophys Res Commun 501: 1060-1067, 2018.
53. Dai ZM, Lv JR, Liu K, Lei XM, Li W, Wu G, Liu XH, Zhu YX, Hao Q and Dai ZJ: The role of microRNA-608 polymorphism on the susceptibility and survival of cancer: A meta-analysis. Aging (Albany NY) 10: 1402-1414, 2018.
54. Yu G, Xiao Q, Ma XP, Chen X, Shi Z, Zhang LY, Chen H, Zhang P, Ding DL, Huang HX, *et al*: miR-492G>C polymorphism (rs2289030) is associated with overall survival of hepatocellular carcinoma patients. Tumour Biol 37: 8961-8972, 2016.
55. Long XD, Huang XY, Yao JG, Liao P, Tang YJ, Ma Y and Xia Q: Polymorphisms in the precursor microRNAs and aflatoxin B1-related hepatocellular carcinoma. Mol Carcinog 55: 1060-1072, 2016.
56. Ji J, Xu M, Tu J, Zhao Z, Gao J, Chen M, Song J, Zhu H, Cheng X, Hui J, *et al*: MiR-155 and its functional variant rs767649 contribute to the susceptibility and survival of hepatocellular carcinoma. Oncotarget 7: 60303-60309, 2016.
57. Wei HX, Tian GX, Song JK, Yang LJ and Wang YP: The association between rs16917496 T/C polymorphism of SET8 gene and cancer risk in Asian populations: A meta-analysis. Biosci Rep 38: BSR20180702, 2018.