Review Article

The Roles of Tumour Suppressor miRNAs in Liver Cancer Progression

Nur Najwa Adlina Mohd Zaid[1], Fazleen Haslinda Mohd Hatta[1], Siti Syairah Mohd Mutalip[1], Ruzianisra Mohamed[1], Mohd Shihabuddin Ahmad Noorden[1]*

Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), 42300 Bandar Puncak Alam, Selangor, Malaysia.

Abstract

Liver cancer is the third leading cause of cancer-related death worldwide. The most prominent type of liver cancer is hepatocellular carcinoma (HCC). One of the most significant factors that contribute to the formation of this cancer is the aberrant microRNA (miRNA) expression in the liver tissue. miRNA is a small non-coding RNA molecule that plays vital roles in various biological processes and has been demonstrated to be the important contributor to the development of liver cancer. miRNA can either act as an oncogenic miRNA or tumour suppressor miRNA depend either they promote or inhibit cancer development in liver cell. The understanding on the factors and molecular mechanism that promote this cancer formation and progression are not completely understood. Thus, this shortcoming will contribute to a lot of drawbacks to the effectiveness of current therapy. This review provides the significant correlation of the tumour suppressor miRNAs' roles in liver cancer development and describes the potential use of miRNA as biomarker and therapeutic RNA drug to treat cancer. Thus, the understanding of molecular mechanisms underlying the initiation and progression of HCC is critical for early diagnosis and developing a new therapeutic strategy.

Keywords: Liver cancer, miRNA, tumour suppressor miRNA, biomarkers, therapeutic agent

Corresponding author
Mohd Shihabuddin Ahmad Noorden, PhD,
Level 11, FF1 Building, Faculty of Pharmacy,
UiTM Puncak Alam, Bandar Puncak Alam, 42300,
Selangor, Malaysia.
budin360@gmail.com

Received 4 July 2021; accepted 29 Oct 2021

Available online: 17 Nov 2021
https://doi.org/10.24191/IJPNaCS.v4.02

1.0 Introduction

Liver cancer is a cancer that develops in the liver due to aberrant proliferation of liver cells and mostly occurs in patients with existing liver disease or cirrhosis. According to databases of cancer worldwide, liver cancer is the third leading cause of cancer-related death in which it is the fifth most common cancer in males while seventh most common cancer in females (1). Hepatocellular carcinoma (HCC) is the most prominent type of liver cancer worldwide which contribute to high mortality rate (2,3).

The development of this cancer can be due to various factors. Chronic Hepatitis B and Hepatitis C infections are the most common cause of liver cancer, which account for about 33% to 60% cases worldwide (2,4,5). Liver cancer that arises due to genetic factors also can account as main contributor of the cancer development (6). Several studies have shown that the development of HCC also can be due to mutation that occurs in SERPINA1, G6PC, SLC37A4, HFE, HMBS, UROD and FAH genes involved in human biological process (2,6). Alterations in genes that are involved in miRNA regulations can also contribute to HCC development and progression (7–9). In the past 5 years, there were clear evidences that demonstrated microRNAs vital roles in promoting the formation and development of liver cancer (10).

The prognosis and diagnosis of this cancer is still limited and unclear which contribute to the weakness and inefficient cancer treatments. High mortality rate worldwide has been associated with the inability to detect the disease at early stage and determine the efficient therapeutic strategies for the disease (4). Hence, diagnosis based on signs and symptoms is important as it could give an accurate diagnose, clear knowledge and a better understanding of the disease. However, the drawback of current inefficient therapeutic strategies in HCC patients could cause a failure to create an effective treatment that specifically inhibit the tumour cell (3). Therefore, the need for a safer and more effective treatments is necessary to provide an accurate information to overcome the weakness of the current therapy. Thus, identification and understanding these miRNAs may open new opportunity for an accurate early diagnose to initiate appropriate treatments.

MicroRNAs have a great potential to be reliable biomarker, target side and therapeutic agent to prevent the HCC development (11). However, the exact functions of miRNAs involved in hepatocellular cancer remain poorly understood and are limited. This review described the role of miRNA as tumour suppressor which emphasizing on their targets and signalling pathways. This review may provide a refined knowledge related to the function of tumour suppressor miRNAs in liver cancer development and it can be useful for future research to support the use of miRNA as potential biomarker and therapeutic agent in cancer treatment.

2.0 MicroRNA

MicroRNA is a small non-coding RNA which plays roles in a variety of biological processes such as biological processes of the body such as cell growth, cell migration, cell invasion, proliferation, differentiation, metabolism and apoptosis (7–9,13,14). They also one of the main contributors in cancer development and progression. A lot of studies have been demonstrated that microRNAs (miRNAs) are capable to regulate gene expression through gene silencing at post-transcriptional process and may have significant effects on biological processes (2,12). However, these biological processes

can also be impaired by the action of miRNAs. In HCC, miRNA expressions are reported either upregulated or downregulated which may promote cancer properties or prevent the cancerous activity by maintaining normal cell properties (14,15).

2.1 Biogenesis of miRNA

Understanding the microRNA biogenesis is important as all types of miRNA have the potential to be developed as therapeutic target. There are several stages involved in the biogenesis of miRNA from nucleus to the cytoplasm. MicroRNA biogenesis starts from the transcription of primary miRNA (pri-miRNA) and undergoes several processes until the formation of mature miRNA which may act on the target mRNA as illustrated in Fig. 1 16. Two main catalytic enzymes that mediate the maturation of pri-miRNA are located in the nucleus (i.e. Drosha) and cytoplasm (i.e. Dicer) (3).

pri-miRNA is transcribed by the RNA polymerase II 8,12. The pri-miRNA structure consists of 5' capped and 3' polyadenylated ends and exist in hairpin-like structure (16-18). Nuclear RNase III Drosha which forms a complex with DGCR8 protein will cause cleavage of the pri-miRNA to form precursor miRNA (pre-miRNA) and it will then be exported into the cytoplasm by Exportin 5 complex to finish the miRNA maturation process (Figure 1) (12,17,19).

Once inside the cytoplasm, miRNA/miRNA* duplex or miRNA double bond is formed through the Dicer catalytic activity (17,18). Helicase enzymes unwind these strands and only the mature miRNA strand (miRNA*) becomes active which is able to cause gene repression by loading into the Argonaute (Ago) protein (17). Then, it will be integrated with the RNA-induced silencing complex (RISC) (12,17). Once loaded into RISC, miRNA is then capable to regulate gene expression (12) through the binding to the target mRNA and interfere translation process (17,18).

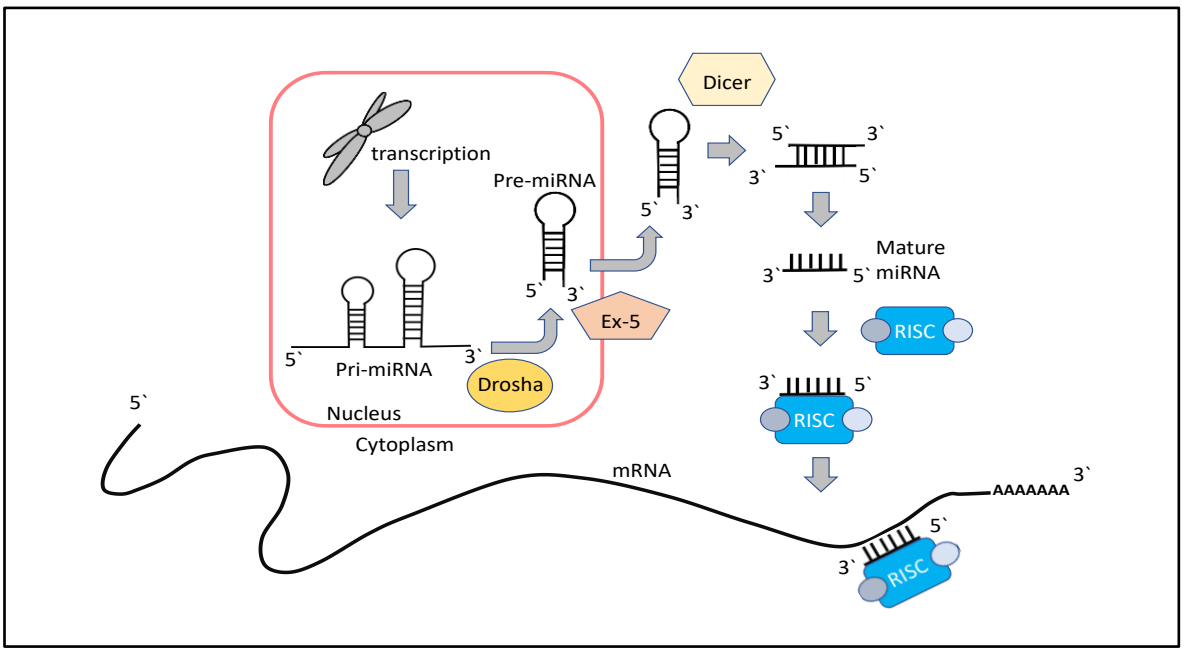

Figure 1: MicroRNA biogenesis pathway (16)

3.0 MicroRNAs in cancer development

Calin et al. reported that more than half of miRNA-encoded genes were located in cancer associated genomic regions(3). Studies have shown miRNA expression is dysregulated in human cancer through various mechanisms, including multi copy number or deletion of miRNA sequences, abnormal production of miRNAs, dysregulated epigenetic changes such as abnormal methylation modification in miRNA genes and defects during miRNA biogenesis (18). MicroRNA contributes to the HCC development by perturbing several signalling pathways in liver such as E-calcium-dependant adhesion (E-cadherin), MNNG HOS transforming gene (MET), v-AKT murine thymoma viral oncogene homolog (Akt), Wnt/β-catenin and others(2). Some researches demonstrated that the changes of key enzymes in the biogenesis of miRNA lead to the progression of HCC (20,21). Moreover, the decreased of these enzyme expressions lead to the formation of cancer cell (22).

In addition, some studies had discovered that patients with chronic hepatitis infection (HBV/HCV) can also be associated with the dysregulation of miRNA expression which could lead to progression of liver cancer (12). In HBV-related HCC, genes that are involved in the critical signalling pathway can be the potential targets of the miRNA (23). Pathways associated with the mechanism of HBV-caused HCC is illustrated in the Figure 2. Various transcription factors can alter the normal expression of miRNA which is capable to regulate the initiation of the cancer. For example, c-Myc is the transcription factor that is mostly upregulated in cancer development and closely associated with the miRNA activities (18).

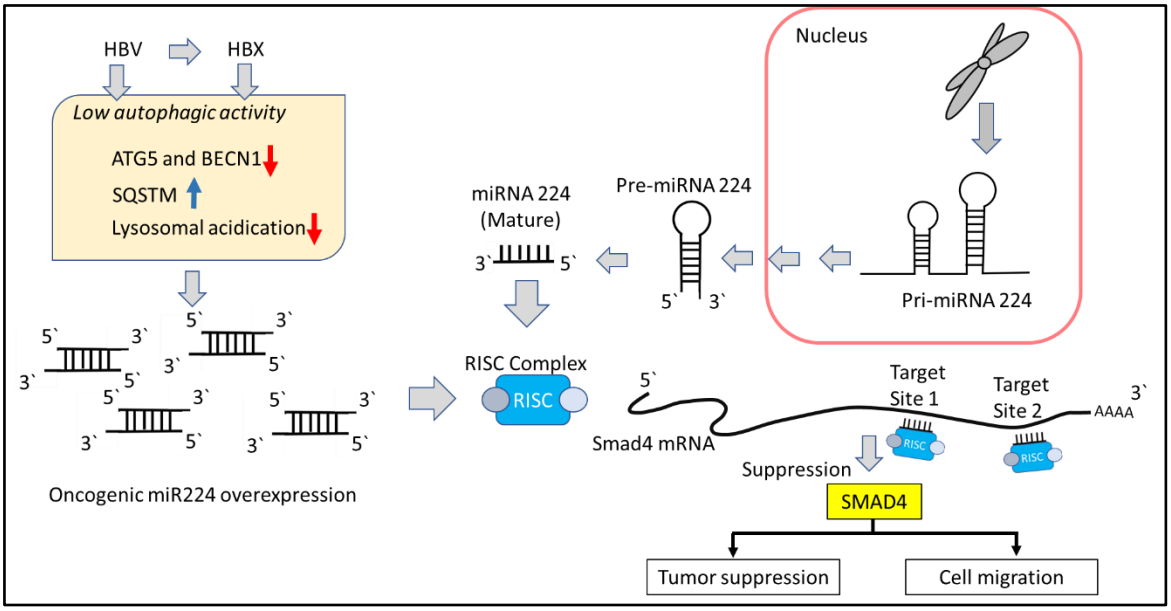

Figure 2: Schematic presentation on how HBV-cause HCC with relation to the MiR224 function. HBV causes a reduction of the autophagy (normal physiological process) activity in the body such as reduced expression of ATG5 and BECN1 protein. Reduce autophagy activity causes upregulation of oncogenic MiR224 which will mature through being loaded into the RISC complex and act on the target mRNA (SMAD4). Thus, the suppression of translation of the SMAD4, then allows the formation of tumour and cell migration (24).

4.0 Type of miRNA involved in liver cancer

MicroRNA can act either as oncogenic miRNA or tumour suppressor miRNA in the liver cancer progression (4). In general, oncogenic miRNAs promote cancer cell development while tumour suppressor miRNA inhibits tumour progression. The initiation and progression of malignancy are mostly due to the interaction of miRNAs with oncogenes and tumour suppressor network (25). Thus, it is important to know the role of miRNAs in proliferation or apoptosis of the cancer cell.

4.1 Oncogenic miRNA

MicroRNA can act as oncogenic miRNA by promoting the overexpression of several oncogenes or by downregulating tumour suppressor genes in the body (2). Most of the cases, the oncogenes are overexpressed which is able to promote cancer initiation. Oncogenic microRNA suppression leads to the inhibition of tumour proliferation due to the return of normal surveillance activity by tumour suppressive genes (17,25).

4.2 Tumour suppressor miRNA

Downregulation of oncogene may be caused by the overexpression of tumour suppressor miRNA and could in turn lead to suppression of the tumorigenesis (2,3) For example, expression of miR-199a as tumour suppressor miRNA leads to the inhibition of oncogene c-Met regulation, the vital oncogene in proliferation of liver cancer cells. However, these tumour suppressor miRNAs are lowly expressed in cancer cells, thus, allowing the upregulation of the oncogene network to promote liver cancer. Figure 3 illustrates the significant tumour suppressor miRNAs that are involved in the initiation of liver cancer (2). For example, in Figure 3, miR-101 can cause inhibition of the Met, RABSA, ATG4D, STMN1, VEGF, c-Fos and ABCA1 which lead to suppression of the tumour cell and promotion of cell apoptosis. This shows that several pathways that can be controlled by one active miRNA.

5.0 Selected tumour suppressor miRNA in liver cancer

This subtopic is reviewing the significant tumour suppressor miRNAs and elaborated their signalling pathways to show their inverse roles in liver cancer.

5.1 MicroRNA-29a

Mir-29a is one of the members from the MiR-29 family and present in the different forms i.e. miR-29b and miR29c (29). These miRNA family members have similar seed region sequence but exhibit different functions in the regulation of target genes (26). Hence, this gives evidence that they are not identical to each other (26). It has been shown that this miRNA expression was downregulated in liver cancer (2,27). Recent study has demonstrated that miR-29a acted as tumour suppressor to inhibit the growth and migration of liver cancer cells (28). There are several cancer-related genes that can be regulated by miR-29a to inhibit tumorigenesis. Osteonectin (SPARC) is one of the miR-29a target genes in HCC (27). When miR-29a is downregulated in the cells, SPACR will be activated via the activation of AKT/mTOR signalling pathway (27,30). AKT/mTOR is involved in the regulation of cell cycle progression. AKT activation is vital and essential in the regulation of tumour cell growth which acts as cell cycle inducer (30). Cell cycle, proliferation and apoptosis could be regulated through the mTOR signalling pathway (27,31). Thus, the proliferation of

the cancer cell can be controlled by increasing the expression of miRNA-29a which will have promising effect on the AKT/mTOR pathway. A previous study has found there was a notably high level of SPARC expression in HCC cells as compared to the normal liver cells (30). This indicates that SPARC is one of the contributors in the cancer progression. Silencing SPARC expression in the HCC can be achieved through the overexpression of miR-29a via AKT inhibition. SPARC contains two binding sites at 3' untranslated region (3'UTR) for the binding of miR-29a which may inhibit SPARC production (30). However, this miRNA is downregulated in liver cancer cell, thus, incapable to inhibit SPARC activity.

Another miR-29a target is CLDN1 which encodes for claudin1. Claudin is a tight junction protein that is capable to regulate the solute movement and is responsible to promote cancer cells migration and metastasis (32). CLDN1 acts as an oncogene in HCC that is activated through the epithelial-mesenchymal transition (EMT) process (33), thus promoting liver cancer metastasis (34). The activation of EMT by CLDN1 is via the expression of the EMT-regulating transcription factors Slug and Zeb1 and potentially increase the cancer cell invasiveness (32). Thus, inhibition of CLDN1 may reduce Zeb1 and Slug expression, and the EMT pathway will not be stimulated, thus, prevent its functions. In HCC, the level of CLDN1 expression is higher in comparison to the normal liver cells. On the other hand, the overexpression of miR-29a causes downregulation of the CLDN1 activity through the binding to 3'UTR of CLDN1 (33).

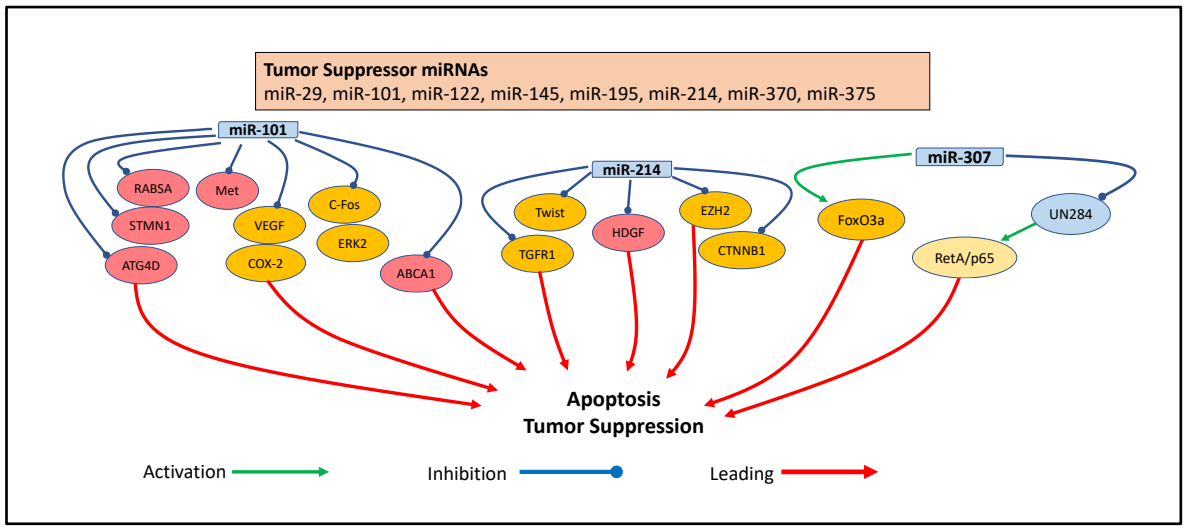

Figure 3: Example of a schematic diagram of tumour suppressive miRNA with its associated target pathway. MiR-29, miR-101, miR-145, miR-375, miR-370, miR-214, miR-122 and miR-195 can perform as tumour suppressor through the activation and inhibition of several gene pathways. For example, MiR-214 inhibits several oncogene pathways including HDGF, EZH2 etc., while MiR-370 activates of the FoxO3a and inhibits UN284 which give signal to tumour suppression and apoptosis (2).

The downregulation of this target will result in the suppression of the HCC progression. CLDN1 is expressed in several human cancers whereby its expression level varies according to cancer cell types. For instance, in cervical cancer, CLDN1 was overexpressed and lead to invasion and migration of cancer while it was downregulated in lung cancer (35,36).

Furthermore, insulin-like growth factor 1 receptor (IGF-1R) can also be regulated by miR-29a in HCC. IGF-1R is a receptor tyrosine kinase that involved in the growth, differentiation and development of cells (37). It functions as an oncogene in the development of cancer (29,38). IGF-1R is actively expressed in liver cancer and its expression is inversely correlated with the expression of miR-29a. Overexpression of miR-29a inhibited the activity of IGF-1R and suppressed the cell growth. Previous studies have demonstrated that inhibition of IGF-1R increased the production of chemokine ligand 5 (CCL5) (37). Hence, the elevation level of CCL5 indicates the overexpression of mir-29a (29). CCL5 is a chemoattractant for $CD8^+$ T lymphocytes which will be recruited and accumulated in the HCC lesions (29,39). Thus, synergic effect of miR-29a and CCL5 will prevent further development of cancer cells (29).

MiR-29a is regulated by xeroderma pigmentosum D (XPD), a subunit of DNA repair or transcription factor II H (TFIIH) to suppress proliferation and migration of HCC cell (40). XPD facilitates the DNA unwinding during the nucleotide repair and acts as tumour suppressor gene (40, 41). When there is a damage in the DNA, XPD will unwind the nucleotide at the damage site through the activation of helicase activity. Liver is an organ that contributes to many metabolic functions in the body and is liable to carcinogenesis that often leads to DNA damage. XPD's ability to repair the damage is reduced in HCC because it was reported to be downregulated in HCC patients 40. The action of XPD in DNA repair is compensated with the p53 pathway in which it will induce the damaged DNA to undergo apoptosis when XPD is mutated (42). However, in the liver cancer patient, this XPD pathway is suspected to be downregulated. A study found that the expression of miR-29a is positively correlated with the expression of XPD (43). Both are downregulated in the patient with liver cancer. XPD acts as tumour suppressor gene in HCC. Hence, XPD can suppress cancer proliferation and migration through the overexpression of miR-29a. Moreover, the level of XPD is low in HepG2 cell line which subsequently promotes cancer cell proliferation (43).

Moreover, alpha-fetoprotein (AFP) expression is significantly high in HCC and is suspected to have a negative correlation with miR-29a expression. AFP is a protein that is mostly elevated during embryogenesis which plays role as a transporter for several metabolic substances, natural killer cell inhibitors and, most importantly in the cell proliferation and tumour growth (44). The level of AFP is reduced as an individual grows older but in HCC patients, the level of AFP is often elevated (45). AFP is believed to perform as a transport protein and biomarker for liver cancer. Previous researches described the influence of c-MYC and AFP to facilitate cancer properties. In the presence of AFP, the binding of c-MYC to the miR-29a may occur and downregulates the miR-29a expression leading to the development of cancer (Fig. 4) (2,44). Hence, miR-29a downregulation leads to cancer cell proliferation and increase expression of AFP which will subsequently increase c-MYC binding to miR-29a.

Multiple target genes can be regulated by a single type of miRNA and one specific

target gene can be suppressed by many types of miRNAs (27). For example, miR-29a can target several proteins including SPARC, CLDN1, IGF-1R and others. Meanwhile, SPARC also can be targeted by other miRNAs such as miR-211 in prostate cancer. Hence, various signalling pathways will be involved through the regulation of miRNA. Table 2 summarised miR-29a's targets in liver cancer.

1

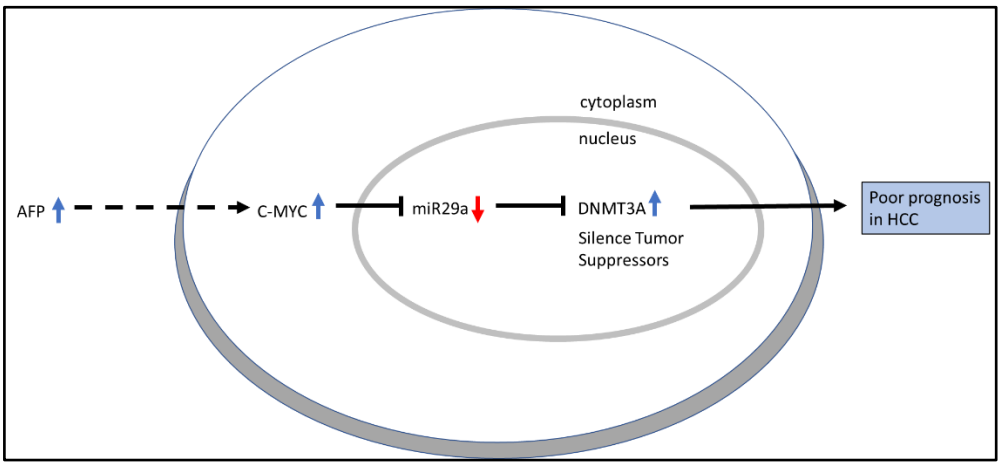

Figure 4 : Schematic diagram on how c-MYC repress miR-29a expression and lead to tumour progression (44).

Table 2: Summary of miR-29a's targets in liver cancer.

Target protein	Expression in cancer	Function	References
Osteonectin (SPARC)	Upregulated	Regulated cell cycle progression through the AKT/mTOR pathway.	27,30
CLDN1 (oncogenes)	Upregulated	Promote cancer cell invasiveness through activation of EMT.	32,33
Insulin-like growth factor 1 receptor (IGF-1R)	Upregulated	Promote cell growth, development and differentiation.	37
XPD (subunit transcription factor II H)	Downregulated	Help DNA unwinding to repair damaged DNA. Inhibit cancer through the overexpression of miR-29a and increase XPD expression.	40,41
Alpha-fetoprotein (AFP)	Upregulated	Metabolic transporter and biomarkers for liver cancer.	2,44

5.2 MicroRNA-145

Several studies had demonstrated that miR-145 is downregulated in liver malignant and acts as tumour suppressor (2,46). The expression of miR-145 in HCC was reported to be linked with the regulation of insulin-like growth factor (IGF) mediator which is insulin receptor substrate-1 (IRS-1) (47). IRS-1 is a protein involved in the signal transmission pathways of insulin and IGF, and capable to mediate the growth of cancer. Previous studies had shown that IRS-1 was overexpressed in the liver cancer (47,48). It is a potential target for miR-145 which have an inverse correlation to each other. The signal transmitted by the IRS-1 will further activate the IGF/AKT and MAPK pathways (47,49). These pathways contribute to the vital role of miRNA in oncogenesis. In HCC, the phosphorylation of AKT is high and effective to induce cellular proliferation and prevent apoptosis (47). FOXO1 is a downstream target of AKT that is capable to promote cell apoptosis by specific gene activation (46,49). Hence, the increased expression of miR-145 causes downregulation of IRS-1 expression and inactivation of AKT pathway. This will lead to an increase in FOXO1 activity and inhibits cancer cell proliferation and cell division.

Histone deacetylase 2 (HDAC2) was foreseen to be solely targeted by miR-145. HDAC2 is an enzyme that is involved in the regulation of various protein expression through the removal of the acetyl group and it associates with the cancer proliferation (49). HDAC2 was found to be abundantly expressed in the HCC patient which indicated the growth of the tumour cell (2,46). The abnormal regulation of HDAC2 induces HCC development via stimulation of cyclin D1, CDK2 and CDK4 (46,49). These are the crucial component that involved in the cell cycle progression. MiR-145 is believed to be a negative regulator in HCC proliferation and has opposite relationship with HDAC2. Restored expression of miR-145 will reduce HDAC2 regulation and inhibit its oncogenic activity in HCC tumorigenesis (46). Disruption of HDAC2 activity contributes to the anti-proliferation effect in the liver cancer. The summary of miR-145's targets in the liver cancer were presented in Table 3.

5.3 MicroRNA-122

MicroRNA 122 shows an incredibly significant effect on the proliferation of liver cancer. MiR-122 is downregulated in liver cancer while it is normally expressed in the normal liver cells (3,50,51). Cyclin G1 can be targeted by miR-122 to prevent cancer progression (50). High expression of the miR-122 caused a reduction in the cyclin G1 activity. Cyclin G1 acts as a promoter for the cell cycle progression (3). The reduction in cyclin G1 activity via the overexpression of miR-122 led to the inhibition of aberrant cell proliferation. Cyclin G1 acts as proto-oncogene on healthy cell and becomes oncogene in HCC development (21). High cyclin G1 expression will downregulate the expression of miR-122 hence increase the occurrence of the tumour. Regulation of p53 protein is controlled by cyclin G1. P53 is a protein that is capable of controlling cell mitosis and induce cell apoptosis. MiR-122 has a repression effect on the p53 protein thus reducing the transcriptional activity of the p53 (51,52). Cyclin G1 is suppressed by the overexpression of miR-122, thus, restores the negative regulation of the p53 at the same time. On the other hand, low miR-122 expression in HepG2 and Hep3B promotes the invasion and growth of the liver tumour (53).

Table 3: Summary of miR-145's targets in liver cancer.

Target	Expression in cancer	Function	References
Insulin receptor substrate-1 (IRS-1)	Upregulated	Signalling pathways of insulin and promote cell growth.	47,48
Histone deacetylase 2 (HDAC2)	Upregulated	Regulate protein expression that is associated with cancer proliferation.	46

IGF-1R is a regulatory mediator in the signalling pathway of cancer which regulates several cellular processes in the body includes homeostasis, differentiation, proliferation and metabolism of cells (52,54). IGF-1R is a complex that comprises the binding of IGF-1 to the transmembrane receptor tyrosine kinase (RTK) (54). MiR-122 downregulation causes an increase in the IGF-1R expression in cancer cells and many studies have reported high amount of IGF-1R in the cancer cell lines. The activation of IGF-1R promotes the proliferation of tumour through the activation of the AKT pathway (55). In HepG2 and Huh-7 cell lines, the abundant secretion of IGF-1R is able to suppress the expression of miR-122 (56). Hence, cancer cell proliferation could be inhibited by suppression of IGF-1R activity that will the restoration of the apoptosis activity.

Wnt/β-catenin is believed to be the most prominent target for miR-122. Wnt/β-catenin is vital in the regulation of various processes including cell migration, differentiation and proliferation. In HCC patients, Wnt/β-catenin signalling pathway is frequently upregulated while the expression of miR-122 is downregulated and this contributes to the cancer progression (57). The regulation of multidrug resistance efflux pump (MDR1) expression is controlled by the Wnt/β-catenin pathway and both are crucial in promoting tumour development. Wnt/β-catenin binds directly to the MDR1 promoter and induces transcription (57). It was reported that the downregulation of miR-122 can cause the activation of Wnt/β-catenin thus affecting the expression of MDR1 (58). However, the increased of miR-122 may repress Wnt/β-catenin signalling pathway and contributes to the inhibition of HCC cell proliferation and induce apoptosis. Hence, this will give the evidence that miR-122 functions as tumour suppressor gene in HCC development (58).

Glucose-6-phosphate dehydrogenase (G6PD) can also be a target for miR-122 binding. It has been shown that miR-122 overexpression caused downregulation of G6PD activity (59). G6PD has an inverse relationship with the expression of miR-122. G6PD is an enzyme that could be activated in the tumour cell which helps nucleotide precursor generation and lipid synthesis (60). Increased G6PD level was observed in the liver cancer patients and this suggested due to the downregulation of miR-122 expression level (59). MiR-122 can bind to the G6PD at three different sites in which two of them are validated as functional sites. The binding will cause repression of the G6PD activity and could possibly prevent cancer cell growth (59). Hence, increased miR-122 expression inhibits G6PD pathway and mediates the suppression of liver tumour. The summary of miR-122's target genes in liver cancer were presented in Table 4.

Table 4: Summary of miR-122's targets in liver cancer

Target	Expression in liver cancer	Function	References
Cyclin-G1	Upregulated	Promote cell cycle proliferation	3,21,51,52
Insulin-like growth factor 1 receptor (IGF-1R)	Upregulated	Regulate several cellular processes such as proliferation and differentiation of cell cycle.	52,54,55
Wnt/β-catenin	Upregulated	Regulation of cell migration, differentiation and proliferation.	57,58
G6PD	Upregulated	Generate precursor nucleotide and lipid synthesis.	59,60

5.4 MicroRNA-199

The microRNA 199 and its family have been shown to play significant roles as tumour suppressor miRNAs in liver cells. MiR-199a and miR-199b have been demonstrated to have notable functions in regulating liver cancer development. One of the targets of miR-199 is CD44, an important glycoprotein that is responsible in the cell adhesion, migration and cell interaction (61). The overexpression of CD44 will mediate the tumour initiation and metastasis. On the other hand, Gao et al. 2015 described the increase expression of miR-199 will able to cause downregulation of CD44, thus preventing cancer initiation and subsequent metastatic process (62). Hence, the level of miR-199 in the human body could potentially be developed as biomarker to predict liver cancer progression.

In HCC, miR-199a is the most significant subtype of miR-199 and it exhibits low expression which contributes to the aberrant proliferation of liver cells (61). The downregulation of this miRNA also demonstrates a high recurrence rate of the cancer cell especially after the surgery. Study from Chao-Hung H *et al*. (61) had shown that MET, mammalian Target of Rifampicin (mTOR) and hypoxia-inducible factor (HIF) are the vital targets of miR-199a in cancer progression (63). Increased expression of miR-199a will interfere with the G1 phase of cell cycle in which it may retard the cell growth and production of various enzymes and protein (63) related to cell proliferation. Hence, the cell is unable to continue growing as several cellular components were unavailable.

High miR-199a expression also capable of mitigating the cancer cells' invasive properties (61). Tumour cells have the capability to metastasize all over the body. They can invade the secondary organs which will contribute to the severe complications to the patient. Hence, miR-199a could potentially be developed as therapeutic agent to prevent liver cancer cells to metastasize to other parts of the body. Furthermore, two active isoforms of miR-199a, miR-199a-5p and miR-199a-3p, have been demonstrated to play important function in liver cancer initiation as shown in Figure 5 (64). miR-199a-3p targets Notch1 and E-Cadherin in liver cells and there is a negative correlation between miR-199a-3p activities on Notch1 or E-Cadherin. In addition, in vitro models demonstrated that miR-199a-3p regulates

E-Cadherin expression through Notch1 direct targeting (64). Low expression of E-Cadherin reduces its adhesive activity and may promote cancer metastasis. However, as reported by Petrova *et al*. (65) sometimes E-Cadherin remains highly expressed on some distal metastases. This in turn promotes the dissociation of the cancer cells and increase the migratory and invasiveness properties to intravasate into the circulatory system. Hence, as suggested by Giovannini *et al*. (64), the restoration of miR-199a-3p may have significant contribution in the complex network of interaction and may inhibit the cancer aggressiveness.

Meanwhile, miR-199a-3p has been highly shown to be downregulated in HCC patients and becomes worse over time due to lack of prognosis (66). Upregulation of mTOR gene in HCC demonstrated low miR-199a-3p expression has a great influence on the proliferation and invasive properties of cancer cells as shown in Fig. 564,66. The activation of mTOR pathways has been shown to cause the progression of liver tumour to be more aggressive as well as shorten the survival rates of patients with HCC (67). Hence, the restoration of this miRNA in the cell could repress mTOR pathways and prevent cancer invasiveness.

Moreover, miR-199a-3p has been revealed to be the most significant isoform of miR-199 in cancer initiation. Besides its activity on the mTOR gene, there are several other genes that are shown to be associated with this miRNA in promoting tumour growth. The phosphorylation of exportin-5 by Extracellular signal-related kinase (ERK) prevents the translocation of the miRNA into the cytoplasm leads to the low miR-199a regulation (68). Its downregulation can enhance tumour cell growth through increased expression of hypoxia inducible factor-1 alpha subunit (HIF1A) and p12 activated kinase 4 (PAK4) protein (66). HIF-1A is involved in the cancer progression and it has high possibility to promote disease recurrent and reduce patient's survival rate (69–71). HIF-1A activity is highly induced under the hypoxia condition in which it represses the adaptive ability of histone demethylase in preserving the cell homeostasis (69). Hence, in HCC patients, their ability to compensate the hypoxic stress is corrupted. Meanwhile, PAK4 has shown a significant elevated level in HCC that is suspected to be the main contributors to the tumour proliferation, invasive and poor survival rates (72,73). Hence, the use of miR-199 as biomarker and therapeutic RNA-based drug in liver cancer can be beneficial. The summary of miR-199 and its related target gene is presented in Table 5.

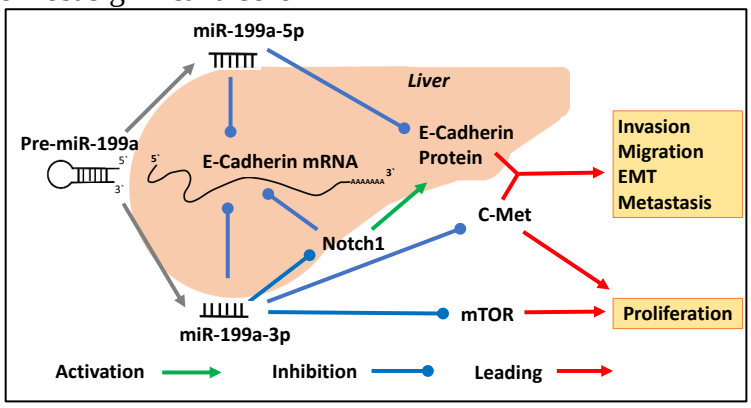

Figure 5: Two active subgroups of miR-199a and their interaction with the different target genes. MiR-199a-5p has ability to enhance expression of E-Cadherin in mitigate cancer metastasis. MiR-199a-3p shows to inhibit mTOR gene in reduce the proliferation of liver cancer (64).

Table 5: Summary of miR-199 and its subtype's target in liver cancer

miR-199 subtypes	Target	Expression in liver cancer	Function	References
miR-199	CD44	Upregulated	Mediate tumour initiation and metastasis through the cell adhesion and cell interaction.	61,62
miR-199a-5p	E-Cadherin	Downregulated	Cell invasion to the other surrounding tissue and cell metastasis	59,60,64,65
miR-199a-3p	Mammalian target of Rifampicin (mTOR)	Upregulated	Tumour cell progression, metastasis and low survival rate	63,64,66,67
	Hypoxia-inducible factor 1 alpha (HIF-1A)	Upregulated	Promote cancer recurrent and reduce survival rate Prevent cell homeostasis	63,66,69–71
	p12 activated kinase 4 (PAK4)	Upregulated	Tumour proliferation, metastasis and poor survival rate	66,72,73

5.5 Let 7

Lethal-7 or known as let 7 was the first miRNA discovered to be downregulated in liver cancer. It is a tumour suppressor miRNA that has the ability to suppress the growth of liver cancer through various mechanisms (74). The loss of let 7 activity or downregulation in let 7 expression contributes to the suppression of liver cancer-associated oncogenes. Let 7 can target many genes that involve in regulating cell cycle, differentiation, migration and invasion. The aberrant regulation of let 7 can be affected by the genetic alterations and perturbation in processing mechanisms (miRNA processing mechanism) which contributes to the aggressive progression of liver cancer 74 .

The association of let 7 in development of HCC is known to associate with the inflammatory responses and most of inflammatory mediators and pathways can modulate tumorigenesis. One of the significant inflammatory pathways that play crucial roles in liver cancer is signal transducer and activator of transcription 3 (STAT3) signalling pathway (75). The activation of this pathway is responsible to initiate the inflammatory responses which result in the development of liver cancer. High expression of let 7 in the liver cells can suppress STAT3 activity, thereby

impedes the cell growth and inflammatory-associated genes. The elevated level of STAT3 in HCC has been demonstrated in various studies. This gene acts as an oncogene where it mediates tumour proliferation and invasion through the activation of interleukin-6 (IL-6) and epidermal growth factor (EGF) (76). Thus, the use of let 7 to control the aberrant cell inflammation can be a promising therapeutic intervention in preventing further cancer progression.

Furthermore, the expression of the RAS gene is correlated with the let 7 mutation. RAS protein is responsible for causing hyper-proliferation of cancer (77). The abnormal expression of let 7 may result in the activation of the RAS gene and leads to progression and invasion of tumour cell (75). Additionally, RAS protein has abundant types of isomers and each of them associates with mutations in different cells (78). Therefore, let 7 expression could facilitate the downregulation of the target gene in order to prevent further complication of cancer cell. Hence, the use of let 7 as a therapeutic agent can be a promising strategy in the future.

Moreover, let 7b has shown a remarkable association with the progression of HCC. Quantitative-PCR (qPCR) showed let 7b expression was highly downregulated in HCC which contributed to the tumorigenesis. The mechanism underlying cancer proliferation is through the upregulation of Wnt/β-catenin and c-Myc genes (79). Wnt/β-catenin gene is an oncogene that is responsible for enhancing cancer proliferation and migration (75,79). The abnormal Wnt/β-catenin activity is noticeable in HCC patients. The overexpression of let 7b in healthy people prevents and blocks the Wnt/β-catenin activation in regard to prevent cancer development. Thus, qPCR result supports the use of let-7b as potential biomarker in HCC patients as its remarkable role in cancer development (79). The ability of let 7b in suppressing the expression of Wnt/β-catenin is extremely useful to be used for early diagnosis and prognosis of liver cancer avoid further complication.

The summary regarding let 7 target genes in regulating cancer development is shown in the Table 6. According to the most relevant previous studies, it can be deduced that the tumour suppressor miRNAs are lowly expressed in cancer cells and inversely inhibit liver cancer development by targeting several oncogenes and stimulating tumour suppressor genes. These targets could be either upregulated or downregulated in cancer development. The review on the related pathways of these miRNAs could help future researchers to conduct research in developing new intervention for liver cancer.

6.0 Future prospective on the roles of miRNA in liver cancer

6.1 MicroRNAs involvement in the diagnosis and prognosis of liver cancer

Identifying miRNAs that could serve as robust diagnostic biomarkers is crucial because the prevalence and death rate for this cancer is rapidly increased over time. miRNA exhibits tissue specificity, thus, can provide better biological markers as compared to the other related HCC biomarkers (2,80–82). In most cases, the tumour were detected at the advanced stage due to the lack of efficient diagnostic biomarkers which will result in poor survival rate (3,82). Therefore, early diagnosis of liver cancer can be achieved by using miRNA as a biomarker and the tumour aggressiveness can be overcame at the early treatments.

Hence, it is remarkably effective to use miRNAs as diagnostic biomarker for liver cancer to improve healthcare management (2,81).

Table 6: Summary of let-7's targets in liver cancer.

Target	Expression in liver cancer	Function	References
STAT3	Upregulated	Initiate inflammatory response, thus, promote cancer proliferation	74–76
RAS gene	Upregulated	Hyper-proliferation of cancer	75,77,78
Wnt/β-catenin	Upregulated	Exaggerated cancer proliferation and migration	75,79

6.2 miRNA as a promising therapeutic agent for treatment of liver cancer

Most cancer cases are detected at the advanced stages, thus make it difficult to provide an effective therapy to prevent cancer metastasis. The therapy such as liver transplantation, radiotherapy and radiosurgery are the only options for the advanced tumour metastasis (2). Exploring the new miRNA target genes as the new potential therapeutic targets can be a useful approach to provide a promising strategy for the cancer treatment (2,3,77,81). The use of miRNA as an anticancer bullet has attracted a lot of attentions since it has high specificity towards the targeted genes involved in cancer development. Hence, the functional studies of diverse miRNA roles in liver cancer is essential as it could provide knowledge to develop a novel potential strategy for the cancer treatment (3,83,84).

There are many ways on how to make use of miRNA knowledge in establishing potential therapy for liver cancer. miRNA biogenesis pathway and any related signalling pathways which response to miRNA activities can be used as potential therapeutic targets (2,77). Furthermore, miRNA itself can be used as a therapeutic agent by introducing exogenous mimic siRNA or expression vector to give continuous effect in liver cancer cell (77,83). Hence, better treatment outcomes can be achieved through miRNA specific binding (2,83,84).

Since a high recurrence rate of liver cancer had been recorded to be attributed to the high morbidity and mortality, therefore the innovative and effective of therapeutics strategies are essential to overcome this big problem. As thoroughly explained above, with an appropriate optimisation, miRNA could be a powerful therapeutic target for liver cancer.

7.0 Conclusion

In this review, we discussed and summarised the latest findings on the roles of tumour suppressor miRNAs and their respected target genes in promoting liver cancer development. This review article further highlighted the significant roles of miRNA in the progression of liver cancer which can be utilised as potential biomarkers for the early detection. Moreover, the advantages of these miRNA in controlling cancer may convinced researchers to explore it as RNA-based drugs or

miRNA-based anticancer agents for therapeutic purposes to reduce the incidence and death rate for this cancer.

Acknowledgement

The authors would like to acknowledge Ministry of Higher Education (MOHE) for Exploratory Research Grant (ERGS) 600-RMI/ERGS 5/3 (52/2012) and Fundamental Research Grant (FRGS) 600-IRMI/FRGS 5/3 (447/2019) for supporting all of our miRNA works. Special thanks to Faculty of Pharmacy, UiTM Puncak Alam Campus for providing the facilities.

Conflict of interest

The authors declare that there is no conflict of interest in the present work.

References

1. Liu CY, Chen KF, Chen PJ. Treatment of liver cancer. Cold Spring Harb Perspect Med. 2015;5(9).

2. Tao J, Jiang L, Chen X. Roles of microRNA in liver cancer. Liver Res. 2018;2(2):61-72.

3. Braconi C, Henry JC, Kogure T, Schmittgen T, Patel T. The role of microRNAs in human liver cancers. Semin Oncol. 2011;38(6):752-763.

4. Li YH, Di CH, Li W, Cai WB, Tan XH, Xu LW, Yang L, Lou GQ, Yan YT. Oncomirs miRNA-221/222 and Tumor Suppressors miRNA-199a/195 Are Crucial miRNAs in Liver Cancer: A Systematic Analysis. Dig Dis Sci. 2016;20:2315-2327.

5. Kiyosawa K, Sodeyama T. Global epidemiology of hepatocellular carcinoma. Nippon Rinsho Japanese J Clin Med. 2001 6;59 Suppl 6(2):13-19.

6. Dragani TA. Risk of HCC: Genetic heterogeneity and complex genetics. J Hepatol. 2010;52(2):252-257.

7. Feng J, Gu X, Liu L, Lu M, Ma X, Cao Y, Jiang R, Wang B, Zhao Q. Prognostic role of MicroRNA-497 in cancer patients: A meta-analysis. J Cancer. 2018;9(18):3334-3342.

8. Thyagarajan A, Shaban A, Sahu RP. MicroRNA-directed cancer therapies: Implications in melanoma intervention. J Pharmacol Exp Ther. 2018;364(1):1-12.

9. Hou G, Xu W, Jin Y, Wu J, Pan Y, Zhou F. MiRNA-217 accelerates the proliferation and migration of bladder cancer via inhibiting KMT2D. Biochem Biophys Res Commun. 2019;519(4):747-753.

10. Bai J, Gao Y, Du Y, Yang X, Zhang X. MicroRNA-300 inhibits the growth of hepatocellular carcinoma cells by downregulating CREPT/Wnt/β-catenin signaling. Oncol Lett. 2019;18(4):3743-3753.

11. Jiang Y, He J, Li Y, Guo Y, Tao H. The Diagnostic Value of MicroRNAs as a Biomarker forHepatocellular Carcinoma: A Meta-Analysis. Hindawi. Biomed Res Int. 2019; 1-14.

12. do Amaral AE, Cisilotto J, Creczynski-Pasa TB, de Lucca Schiavon L. Circulating miRNAs in nontumoral liver diseases. Pharmacol Res. 2018;128:274-287.

13. Nair VS, Pritchard CC, Tewari M and Ioannidis JPA. Design and Analysis for Studying microRNAs in Human Disease: A Primer on -Omic Technologies. Am J Epidemiol. 2014; 180(2): 140–152.

14. Nair VS, Pritchard CC, Tewari M, Ioannidis JPA. Design and analysis for studying microRNAs in human disease: A primer on-omic technologies. Am J Epidemiol. 2014;180(2):140-152.

15. Qiu L, Zhang GF, Yu L, Wang HY, Jia XJ, Wang TJ. Novel oncogenic and chemoresistance-inducing functions of resistin in ovarian cancer cells require miRNAs-mediated induction of epithelial-to-mesenchymal transition. Sci Rep. 2018;8(1):1-10.

16. Biggar KK, Storey KB. Insight into post-transcriptional gene regulation: Stress-responsive microRNAs and their role in the environmental stress survival of tolerant

animals. J Exp Biol. 2015;218(9):1281-1289.

17. Hung CH, Chiu YC, Chen CH, Hu TH. MicroRNAs in hepatocellular carcinoma: Carcinogenesis, progression, and therapeutic target. Biomed Res Int. 2014; 2014: 486407.

18. Peng Y, Croce CM. The role of microRNAs in human cancer. Signal Transduct Target Ther. 2016 (1):15004. 1-9.

19. Frixa T, Donzelli S, Blandino G. Oncogenic MicroRNAs: Key players in malignant transformation. Cancers (Basel). 2015;7(4):2466-2485.

20. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne). 2018; (402): 1-12.

21. Chu R, Mo G, Duan Z, Huang M, Chang J, Li X, Liu P. MiRNAs affect the development of hepatocellular carcinoma via dysregulation of their biogenesis and expression. Cell Commun Signal. 2014;12(1):1-18.

22. Wu K, He J, Pu W, Peng Y. The Role of Exportin-5 in MicroRNA Biogenesis and Cancer. Genomics, Proteomics Bioinforma. 2018;16(2):120-126.

23. Wang GY, Dong FL, Xu ZY, Sharma S, Hu XT, Chen DF, Zhang LM, Zhang JP, Dong QH. MicroRNA profile in HBV-induced infection and hepatocellular carcinoma. BMC Cancer. 2017;17(1):1-11.

24. Lan SH, Wu SY, Zuchini R, Lin XZ, Su IJ, Tsai TF, Lin YJ, Wu CT, Liu HS. Autophagy-preferential degradation of MIR224 participates in hepatocellular carcinoma tumorigenesis. Autophagy. 2014;10(9):1687-1689.

25. Chiara Raggi, Pietro Invernizzi JBA. Impact of microenvironment and stem-like plasticityin cholangiocarcinoma: Molecular networks and biological concepts. ICSID Rev. 2015;62:198-207.

26. Kriegel AJ, Liu Y, Fang Y, Ding X, Liang M. The miR-29 family: Genomics, cell biology, and relevance to renal and cardiovascular injury. Physiol Genomics. 2012;44(4):237-244.

27. Wang JY, Zhang Q, Wang DD, Yan W, Sha HH, Zhao JH, Yang SJ, Zhang HD, Hou JC, Xu HZ, He YJ, Hu JH, Zhong SL, Tang JH. MiR-29a: A potential therapeutic target and promising biomarker in tumors. Biosci Rep. 2018;38(1):1-21.

28. Lin LL, Wang W, Hu ZY, Wang LW, Chang J, Qian HG. Negative feedback of miR-29 family TET1 involves in hepatocellular cancer. Med Oncol. 2014;31(12):1-9.

29. Kwon JJ, Factora TD, Dey S, Kota J. A Systematic Review of miR-29 in Cancer. Mol Ther - Oncolytics. 2019;12:173-194.

30. Zhu XC, Dong QZ, Zhang XF, Deng B, Jia HL, Ye QH, Qin LX, Wu XZ. microRNA-29a suppresses cell proliferation by targeting SPARC in hepatocellular carcinoma. Int J Mol Med. 2012; 30(6):1321-1326.

31. Zhu M, Wang M, Yang F, Tian Y, Cai J, Yang H, Fu H, Mao F, Zhu W, Qian H, Xu W. miR-155-5p inhibition promotes the transition of bone marrow mesenchymal stem cells to gastric cancer tissue derived MSC-like cells via NF-κB p65 activation. Oncotarget. 2016;7(13):16567-16580.

32. Shaya Mahati, Lei Xiao, Ying Yang, Rui Mao YB. miR-29a suppresses growth and migration of hepatocellular carcinoma by regulating CLDN1. Biochem Biophys Res Commun. 2017 ;486(3):732-737.

33. Shaya Mahati, Lei Xiao, Ying Yang, Rui Mao YB. miR-29a suppresses growth and migration of hepatocellularcarcinoma by regulating CLDN1. Biochem Biophys Res Commun. 2017 ;486(3):732-737.

34. Pope JL, Ahmad R, Bhat AA, Washington MK, Singh AB, Dhawan P. Claudin-1 overexpression in intestinal epithelial cells enhances susceptibility to adenamatous polyposis coli-mediated colon tumorigenesis. Mol Cancer. 2014;13(1):1-13.

35. Sun B sheng, Yao Y qun, Pei B xiang, Zhang Z fa, Wang C li. Claudin-1 correlates with poor prognosis in lung adenocarcinoma. Thorac Cancer. 2016;7(5):556-563.

36. Zhang WN, Li W, Wang XL, Hu Z, Zhu D, Ding WC, Liu D, Li KZ, Ma D, Wang H. CLDN1 expression in cervical cancer cells is related to tumor invasion and metastasis.

Oncotarget. 2016;7(52):87449-87461.

37. Wang X, Liu S, Cao L, Zhang T, Yue D, Wang L, Ping Y, He Q, Zhang C, Wang M, Chen X, Gao Q, Wang D, Zhang Z, Wang F, Yang L, Li J, Huang L, Zhang B, Zhang Y. miR-29a-3p suppresses cell proliferation and migration by downregulating IGF1R in hepatocellular carcinoma. Oncotarget. 2017;8(49):86592-86603.

38. Xiao Y, Tian Q, He J, Huang M, Yang C, Gong L. MiR-503 inhibits hepatocellular carcinoma cell growth via inhibition of insulin-like growth factor 1 receptor. Onco Targets Ther. 2016;9:3535-3544.

39. Aldinucci D, Colombatti A. The inflammatory chemokine CCL5 and cancer progression. Mediators Inflamm. 2014;2014:1-19.

40. Xiao Z, Wang Y, Ding H. XPD suppresses cell proliferation and migration via miR-29a-3p-Mdm2/PDGF-B axis in HCC. Cell Biosci. 2019;9(1):1-16.

41. Yuan T, Deng S, Liu H, Liu M, Chen P. Relationship between XRCC1 and XPD polymorphisms and the risk of the development of hepatocellular carcinoma: A case-control study. Exp Ther Med. 2012;4(2):285-290.

42. Hao Ding, Jiang-jing Xu, Yuan Huang, Fang-teng Dua JZ. XPD could suppress growth of HepG2 . 2 . 15 and down-regulate the expression of hepatitis B virus x protein through P53 pathway. Biochem Biophys Res Commun. 2012;419(4):761-767.

43. Zheng JF, Li LL, Lu J, Yan K, Guo WH, Zhang JX. XPD functions as a tumor suppressor and dysregulates autophagy in cultured HepG2 cells. Med Sci Monit. 2015;21:1562-1568.

44. Parpart S, Roessler S, Dong F, Rao V, Takai A, Ji J, Qin LX, Ye QH, Jia HL, Tang ZY, Wanget XW. Modulation of miR-29 expression by alpha-fetoprotein is linked to the hepatocellular carcinoma epigenome. Hepatology. 2014;60(3):872-883.

45. Bai DS, Zhang C, Chen P, Jin SJ, Jiang GQ. The prognostic correlation of AFP level at diagnosis with pathological grade, progression, and survival of patients with hepatocellular carcinoma. Sci Rep. 2017;7(1):1-15.

46. Noh JH, Chang YG, Kim MG, Jung KH, Kim JK, Bae HJ, Eun JW, Shen Q, Kim SJ, Kwon SH, Park WS, Lee JY, Nam SW. MiR-145 functions as a tumor suppressor by directly targeting histone deacetylase 2 in liver cancer. Cancer Lett. 2013;335(2):455-462.

47. Yin Y, Yan ZP, Lu NN, Xu Q, He J, Qian X, Yu J, Guan X, Jiang BH, Liu LZ. Downregulation of miR-145 associated with cancer progression and VEGF transcriptional activation by targeting N-RAS and IRS1. Biochim Biophys Acta - Gene Regul Mech. 2014;1829(2):239-247.

48. Sakurai Y, Kubota N, Takamoto I, Obata A, Iwamoto M, Hayashi T, Aihara M, Kubota T, Nishihara H, Kadowaki T. Role of insulin receptor substrates in the progression of hepatocellular carcinoma. Sci Rep. 2017;7(1):1-33.

49. Xing A, Wang B, Shi D, Zhang XF, Gao C, He XQ, Liu WJ, Gao P. Deregulated expression of miR-145 in manifold human cancer. Exp Mol Pathol. 2013; 95(1):91-97.

50. Tsai WC, Hsu S Da, Hsu CS, Lai TC, Chen SJ, Shen R, Huang Y, Chen HC, Lee CH, Tsai TF, Hsu MT, Wu JC, Huang HD, Shiao MS, Hsiao M, Tsou AP. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J Clin Invest. 2012;122(8):2884-2897.

51. Bandiera S, Pfeffer S, Baumert TF, Zeisel MB. MiR-122 - A key factor and therapeutic target in liver disease. J Hepatol. 2015;62(2):448-457.

52. Nakao K, Miyaaki H, Ichikawa T. Antitumor function of microRNA-122 against hepatocellular carcinoma. J Gastroenterol. 2014;49(4):589-593.

53. Lou G, Song X, Yang F, Wu S, Wang J, Chen Z, Liu Y. Exosomes derived from MIR-122-modified adipose tissue-derived MSCs increase chemosensitivity of hepatocellular carcinoma. J Hematol Oncol. 2015;8(1):1-11.

54. Thalyana SV, Slack FJ. MicroRNAs and their roles in aging. J Cell Sci. 2012;125(1):7-17.

55. El Tayebi HM, Abdelaziz AI. Epigenetic

regulation of insulin-like growth factor axis in hepatocellular carcinoma. World J Gastroenterol. 2016;22(9):2668-2677.

56. Xu Y, Huang J, Ma L, Shan J, Shen J, Yang Z, Liu L, Luo Y, Yao C, Qian C. MicroRNA-122 confers sorafenib resistance to hepatocellular carcinoma cells by targeting IGF-1R to regulate RAS/RAF/ERK signaling pathways. Cancer Lett. 2016;371(2):171-181.

57. Liu LJ, Xie SX, Chen YT, Xue JL, Zhang CJ, Zhu F. Aberrant regulation of WNT signaling in hepatocellular carcinoma. World J Gastroenterol. 2016;22(33):7486-7499.

58. Cao F, Yin LX. miR-122 enhances sensitivity of hepatocellular carcinoma to oxaliplatin via inhibiting MDR1 by targeting Wnt/β-catenin pathway. Exp Mol Pathol. 2019;106:34-43.

59. Barajas JM, Reyes R, Guerrero MJ, Jacob ST, Motiwala T, Ghoshal K. The role of miR-122 in the dysregulation of glucose-6-phosphate dehydrogenase (G6PD) expression in hepatocellular cancer. Sci Rep. 2018;8(1):1-31.

60. Mele L, La Noce M, Paino F, Regad T, Wagner S, Liccardo D, Papaccio G, Lombardi A, Caraglia M, Tirino V, Desiderio V, Papaccio F. Glucose-6-phosphate dehydrogenase blockade potentiates tyrosine kinase inhibitor effect on breast cancer cells through autophagy perturbation. J Exp Clin Cancer Res. 2019;38(1):1-24.

61. Chao-Hung H, Yi-Chun C, Chien-Hung C, Tsung-Hui H. MicroRNAs in Hepatocellular Carcinoma: Carcinogenesis, Progression, and Therapeutic Target. Biomed Res Int. 2014; 2014: 486407.

62. Gao Y, Feng Y, Shen JK, Lin M, Choy E, Cote GM, Harmon DC, Mankin HJ, Hornicek FJ, Duan Z. CD44 is a direct target of miR-199a-3p and contributes to aggressive progression in osteosarcoma. Sci Rep. 2015;5(1):1-9.

63. Fornari F, Milazzo M, Chieco P, Negrini M, Calin G, Grazi G, Pollutri D, Croce C, Bolondi L, Gramantieri L. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res. 2015;70(12):5184-5193.

64. Giovannini C, Fornari F, Dallo R, Gagliardi M, Nipoti E, Vasuri F, Coadă CA, Ravaioli M, Bolondi L, Gramantieri L. MiR-199-3p replacement affects E-cadherin expression through Notch1 targeting in hepatocellular carcinoma. Acta Histochem. 2018;120(2):95-102.

65. Petrova YI, Schecterson L, Gumbiner BM. Roles for E-cadherin cell surface regulation in cancer. Mol Biol Cell. 2016;27(21):3233-3244.

66. Callegari E, D'Abundo L, Guerriero P, Simioni C, Elamin BK, Russo M, Cani A, Bassi C, Zagatti B, Gacomelli L, Blandamura S, Moshiri F, Ultimo S, Frassoldati A, Altavilla G, Gramantieri L, Neri LM, Sabbioni S, Negrini M. miR-199a-3p Modulates MTOR and PAK4 Pathways and Inhibits Tumor Growth in a Hepatocellular Carcinoma Transgenic Mouse Model. Mol Ther - Nucleic Acids. 2018;11:485-493.

67. Ferrín G, Guerrero M, Amado V, Rodríguez-Perálvarez M, De la Mata M. Activation of mTOR signaling pathway in hepatocellular carcinoma. Int J Mol Sci. 2020;21(4):1266: 1-16.

68. Sun HL, Cui R, Zhou JK, Teng KY, Hsiao YH, Nakanishi K, Fassan M, Luo Z, Shi G, Tili E, Kutay H, Lovat F, Vicentini C, Huang HL, Wang SW, Kim T, Zanesi N, Jeon YJ, Lee TJ, Guh JH, Hung MC, Ghoshal K, Teng CM, Peng Y, Croce CM. ERK Activation Globally Downregulates miRNAs through Phosphorylating Exportin-5. Cancer Cell. 2016;30(5):723-736.

69. Chen C, Lou T. Hypoxia inducible factors in hepatocellular carcinoma. Oncotarget. 2017;8(28):46691-46703.

70. De Matteis S, Scarpi E, Granato AM, Vespasiani-Gentilucci U,Barba GL, Foschi FG, Bandini E, Ghetti M, Marisi G, Cravero P, Gramantieri L, Cucchetti A, Ercolani G, Santini D, Frassineti GL, Faloppi L, Scartozzi M, Cascinu S, Casadei-Gardini A. Role of SIRT-3, p-mTOR and HIF-1α in Hepatocellular Carcinoma Patients Affected by Metabolic Dysfunctions and in Chronic Treatment with Metformin. Int J Mol Sci. 2019;20(6) 1503.

71. Ambade A, Satishchandran A, Saha B, Gyongyosi B, Lowe P, Kodys K, Catalano D, Szabo G. Hepatocellular carcinoma is accelerated by NASH involving M2 macrophage polarization mediated by hif-1αinduced IL-10. Oncoimmunology.

2016;5(10) e1221557. 1-13.

72. Won SY, Park JJ, Shin EY, Kim EG. PAK4 signaling in health and disease: defining the PAK4–CREB axis. Exp Mol Med. 2019;(51) 1-9.

73. Lu SX, Zhang CZ, Luo RZ, Wang CH, Liu LL, Fu J, Zhang L, Wang H, Xie D, Yun JP. Zic2 promotes tumor growth and metastasis via PAK4 in hepatocellular carcinoma. Cancer Lett. 2017;402:71-80.

74. Chirshev E, Oberg KC, Ioffe YJ, Unternaehrer JJ. Let - 7 as biomarker, prognostic indicator, and therapy for precision medicine in cancer . Clin Transl Med. 2019;8(1):24. 1-14.

75. Huan L, Liang LH, He XH. Role of microRNAs in inflammation-associated liver cancer. Cancer Biol Med. 2016;13(4):407-425.

76. Lee C, Cheung ST. STAT3: An emerging therapeutic target for hepatocellular carcinoma. Cancers (Basel). 2019;11(11).

77. Callegari E, Gramantieri L, Domenicali M, D'Abundo L, Sabbioni S, Negrini M. MicroRNAs in liver cancer: A model for investigating pathogenesis and novel therapeutic approaches. Cell Death Differ. 2015;22(1):46-57.

78. Hobbs GA, Der CJ, Rossman KL. RAS isoforms and mutations in cancer at a glance. J Cell Sci. 2016;129(7):1287-1292.

79. Wang Y, Mo Y, Wang L, Su P, Xie Y. Let-7b contributes to hepatocellular cancer progression through Wnt/β-catenin signaling. Saudi J Biol Sci. 2018;25(5):953-958.

80. Long X-D, Tang W-Z, Lu J, Huang XY, Yao JG, Zhang TQ, Wang XZ, Su QY, Luo CY, Wu XM, Wang C, Zeng LX, Xia Q, Ma Y. The Diagnostic and Prognostic Potential of MicroRNAs for Hepatocellular Carcinoma. In: Hepatocellular Carcinoma - Advances in Diagnosis and Treatment. IntechOpen Book Series; 2017; (5) 103-128.

81. Shen S, Lin Y, Yuan X, Shen L, Chen J, Chen L, Qin L, Shen B. Biomarker MicroRNAs for Diagnosis, Prognosis and Treatment of Hepatocellular Carcinoma: A Functional Survey and Comparison. Sci Rep. 2016; 6 (38311) 1-21.

82. Jin Y, Wong YS, Goh BKP, Chan CY, Cheow PC, Chow PKH, Lim TKH, Goh GBB, Krishnamoorthy TL, Kumar R, Ng TP, Chong SS, Tan HH, Chung AYF, Ooi LLPJ, Chang JPE, Tan CK, Lee CGL. Circulating microRNAs as Potential Diagnostic and Prognostic Biomarkers in Hepatocellular Carcinoma. Sci Rep. 2019;9(1):1-12.

83. Bimonte S, Leongito M, Barbieri A, Vecchio V, Falco M, Giudice A, Palaia R, AlbinoV, Giacomo RD, Petrillo A, Granata V, Izzo1 F. The Therapeutic Targets of miRNA in Hepatic Cancer Stem Cells. Hindawi. Stem Cell International. 2016; (2016) 1-10.

84. Onishi M, Ochiya T, Tanaka Y. MicroRNA and liver cancer. Cancer Drug Resist. Cancer Drug Resist 2020;3:385-400.