

miR-122 and miR-21 as clinical biomarkers in hepatocellular carcinoma: A review

Nishant Kumar¹, Priyvar Choudhary¹, Narotam Sharma², Alok Tripathi³, Nishesh Sharma^{1*}

¹School of Applied and Life Sciences, Uttarakhand University, Premnagar, Dehradun, Uttarakhand-248007, India.

²DNALabs Dehradun, Uttarakhand 248001, India.

³Aavya Life Science Pvt. Ltd., Geeta Nagar, Kanpur, Uttar Pradesh-208025, India.

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ABSTRACT

Molecular diagnostics represent techniques implemented to search and identify, biological markers located in genome or cellular pool. Molecular markers are looked upon an indispensable tool for diagnosis of wide range of diseases. Hepatocellular carcinoma (HCC) development is attributed to liver diseases and risk factors such as, hepatitis-C virus, hepatic abnormalities, cirrhosis, and metabolic disorders causing liver damage. A challenge for available treatment methods for HCC is lack of early stage diagnosis. Micro-RNAs (mi-RNAs) are regulatory non-coding RNA, smaller in size having a significantly key role in the modulation of gene expression, post-transcriptional modification of messenger RNA (mRNA), and controlling cell progression, differentiation, and apoptosis. The serum and plasma of HCC patients contain dysregulated mi-RNAs during migration, invasion, and development of HCC. It is evident that mi-RNA expression is increased or decreased in specific cancer cell lines and tissues, and thus it could be considered as disease biomarker for the investigation of HCC. In this review, the functional level of mi-RNAs (miR-122 and miR-21), its molecular aspects, and gene regulation is reviewed to identify their role as potential biomarkers for HCC diagnosis and treatment.

1. INTRODUCTION

Non-coding RNAs are class of functional regulatory non-protein coding RNAs and have potential role in different biomedical applications [1]. Scientific studies related to non-coding RNAs have expand impetus continuously from past to current time showing its ability in the area of gene expression modulation and control in cellular innate and adaptive immunity [2]. Micro-RNAs (mi-RNAs) are members of the small non-coding RNA family, have an important role in regulation of gene expression. They are non-coding regulatory RNA, small in size of approx ~22 nucleotides belongs to class of endogenous post-transcriptional regulators known to interact with expression of other genes, commonly identified as target genes [3]. Interaction of mi-RNA with target genes generally results in suppression of gene expression of target genes, by the means of translational repression, messenger RNA (mRNA) cleavage, and deadenylation [4]. Mi-RNAs and their associated proteins are found in abundance in the ribo-nucleoprotein complexes in the cells. As per the research report of different studies, it is proved that mi-RNAs are found in different biologically important

body fluids such as serum, plasma, urine, peritoneal cavity fluid, cerebrospinal fluid, and fluid of follicles [5,6]. These mi-RNAs released in extracellular fluids and cells act as potential biomarkers for several diseases and cancer [7].

Now, it is evident from research result that mi-RNAs have role in the development of the several types of cancer and it is due to the dysregulation of the different oncogenic and tumor suppressor mi-RNAs. Each transcribed mi-RNA targets specific mRNA in the cytoplasm and performs regulatory mechanisms in a complex manner by formation of mi-RNAs-based RNA silencing complexes (RISCs) [Figure 1] [8]. The pre-transcribed mi-RNAs (pri-mi-RNAs) are dysregulated by unregulated expression of DROSHA/DGCR8 microprocessor proteins by either mutation or epigenetic factors [9]. The mi-RNAs may be upregulated or downregulated in HCC and perform transverse actions on tumor progression. The upregulated mi-RNAs act as onco-miRs, while the downregulated mi-RNAs act as a tumor suppressor.

The clinical significance of mi-RNA was identified in the therapy of several diseases. Mi-RNA antagonists and mi-RNA mimics are two approaches involved in utilization of mi-RNA molecule as therapeutic agents. Mi-RNA antagonists represent antisense RNA approach in which chemically modified mi-RNA are introduced to bind to target mi-RNA, that is, over expressed mi-RNAs in diseased cell type results in formation of irreversible mi-RNA duplex which is ultimately degraded. Mi-RNA antagonists find their application when target mi-

*Corresponding Author:

Dr. Nishesh Sharma

Associate Professor, Department of Biotechnology,

School of Applied and Life Sciences, Uttarakhand University,

Dehradun, Uttarakhand-248007, India.

E-mail: nishesh21@gmail.com

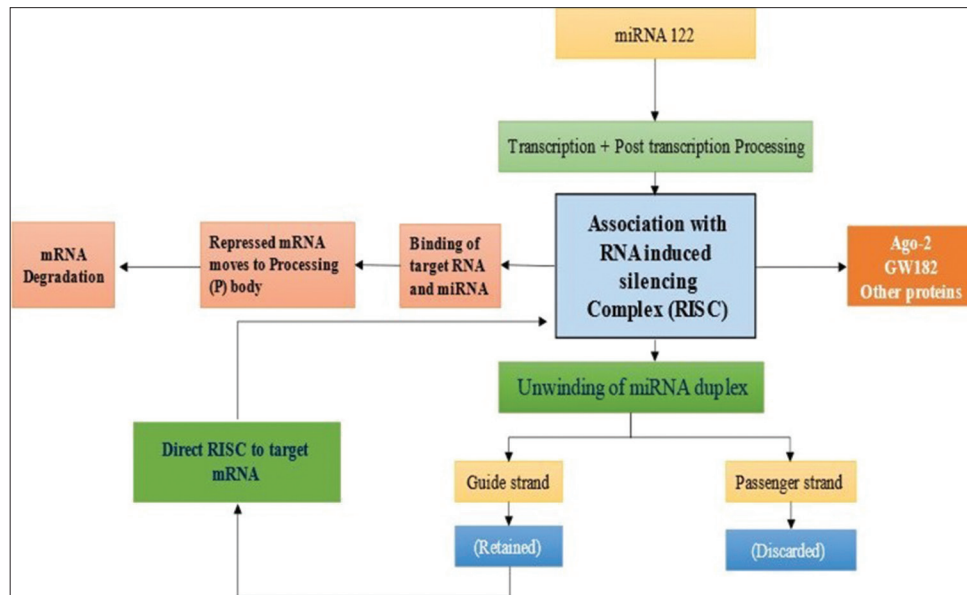


Figure 1: Summary of miR-122 cellular expression and processing [8-10].

RNA acquires a gain of function in diseased cell type. On the other hand, mi-RNA mimics come into play when target mi-RNA encounters loss of function. Mi-RNA mimics are introduced into diseased cell type to restore normal function. Advantages associated with utilization of mi-RNA as therapeutic molecules include their specific nature, tolerance in normal tissues, and few delivery issues as compared to protein encoding plasmid vector.

At present, several mi-RNAs have been discovered with relevance to the different important diseases. Here, focuses are made to the mi-RNAs having important role in the genesis and progression of liver cancer. In this regard, two main mi-RNAs selected are miR-122 and miR-21, details are as follows.

1.1. Mi-RNA 122 (miR-122)

Liver is considered as one of the most vital organ of mammalian body system. This organ has ability of self-regeneration of hepatic cells as well as it is actively involved in metabolic activities. Several mi-RNA has been accounted to its specific key role in biological activities of liver [Figure 2] [10]. Among liver related mi-RNAs, miR-122 is most abundant prominent biomarker for its biological activities and comprises about 52% of the total hepatic mi-RNAs. It is also among the highest expressed mi-RNA in liver cell with approximate 66,000 copies per hepatic cell. In humans, gene encoding miR-122 is specifically located on the chromosome number 18, has important role in several liver functions along with reported anti-inflammatory activity [11].

Studies suggested the multifunctional role of miR-122 in liver associated diseases and also involved in life cycle of pathogens infecting liver cells. It has been reported that down regulated miR-122 expression in liver fibrosis is due to prolonged chronic alcohol consumption. Regulation of inflammation, diet-induced obesity, cholesterol and iron metabolism, and fat metabolism are other clinical implications involving miR-122 [Figure 2]. However, now a days, most of the studies emphasized the crucial role of miR-122 in the development of liver cancer [12,13]. Figure 1 summarizes the outline of cellular processing of miR122.

1.2. Mi-RNA 21 (miR-21)

MiR-21 is the first time reported and discovered mi-RNA among

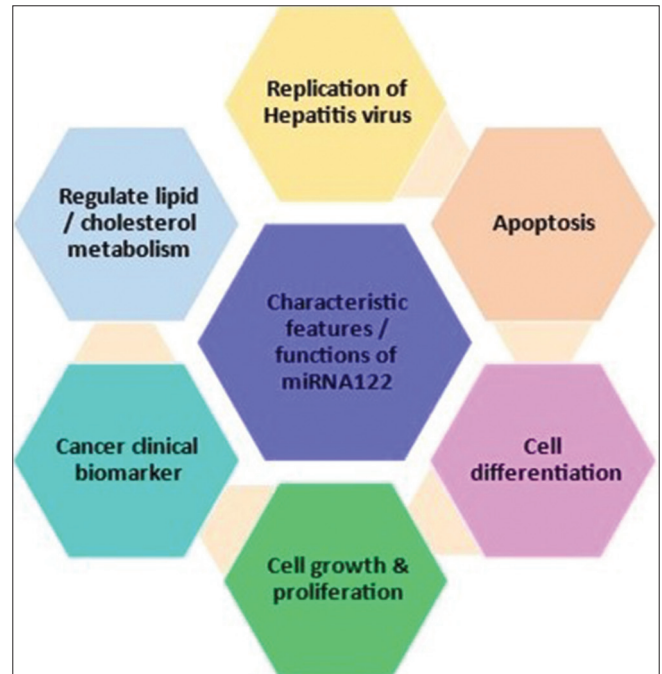


Figure 2: Major cellular function of miR-122 [12,13].

all mi-RNAs identified, also known as has-miR21, miR-21 which is highly conserved and abundant micro-RNA. It is reported to be expressed in several cell types and is involved in several biologically important functions related to health and diseases [Figure 3]. The most promising application of miR-21 is in clinical diagnostics as a potential biomarker in early cancer diagnosis. The three main target of miR-21 are programmed cell death 4, tropomyosin 1 and phosphatase and tensin homolog (PTEN) [14-17]. The miR-21 is located in vacuole membrane protein-1 present on chromosome-7. High concentration of miR-21 supports its easy detection within different biological fluids named serum, urine, plasma, etc. The stability of miR-21 in body fluids supports utilization of miR-21 as a biomarker. The study conducted have reported miR-21 to act as oncogene and when over-

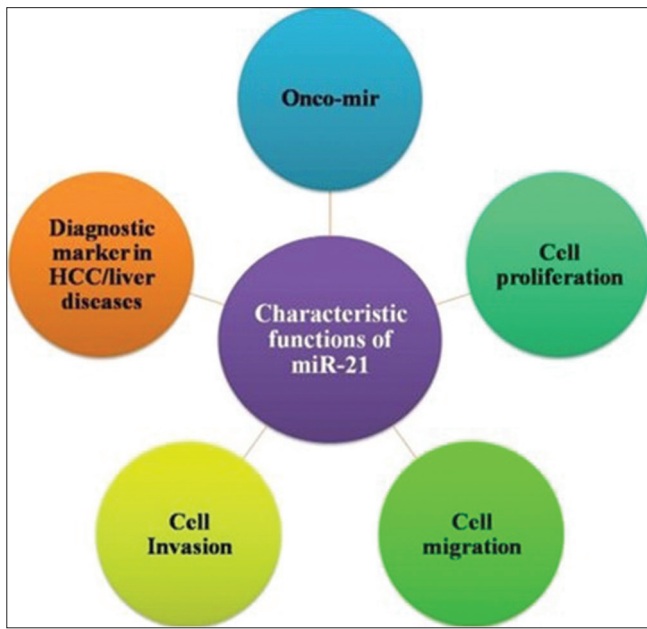


Figure 3: Major cellular function of miR-21 [15,16,18,19].

expressed results in malignancy [18]. Mi-RNA upregulation by cytokines indicates their potential role as inflammatory response. It is found in various reports that miR-21 has involved in neoplastic as well as non-neoplastic diseases. Jenike and Halushka have provided a detailed review including expression of miR-21 in sixteen non-neoplastic diseases and 13 neoplastics [19]. Besides being a highly expressed mi-RNA, miR-21 is also expressed in large numbers of cell types with extremely high concentration in monocytes, macrophages, and dendritic cells. However, high level of expression of miR-21 in several cell types appears as a challenge for clinical application of miR-21 as a specific biomarker. Hence, relevant researches are essential requirements for the establishment of miR-21 as potential biomarker or as regulatory molecule.

2. BIOMEDICAL APPLICATIONS

2.1. Mi-RNA-122 in Liver Cancer Diagnosis

Studies conducted have established miR-122 to exhibit potential to be utilized as biomarker in liver cancer diagnosis. The diagnostic potential application is attributed to differential gene expression of miR-122 in cancerous and non-cancerous cells. Dai *et al.*, studied the applicability of miR-122 in the diagnosis as well as prognosis of cancer with specific reference to hepatocellular carcinoma (HCC). This study reported the elevated expression of miR-122 in the case of HCC in comparison to miR-122 expression in normal cells. As per the research result, it is also recommended that combination of miR-122 with alpha-feto protein can further enhance the diagnostic efficiency to utilize mi-RNA expression as biomarker for cancer diagnosis [20]. Another specifically studied clinical application of miR-122 is in Hepatitis C virus (HCV) infection. As HCV replication requires interaction of miRNA-122 with 5' non-coding region of the virus, so decreased miR-122 expression has been directly related to reduction in replication of HCV [21]. The durable schematic representation regarding cell culture for HCV infection is limited only to the researcher using specific clones for HCV cell culture and cultured cells derived from human specific hepatoma cell line Huh7. Human specific hepatic cell lines specific to lentiviral vector showed a higher expression pattern of miR-122 comparable to endogenous expression pattern in Huh7 cell line.

Cells of Hep3B cell line gradually corresponded miR-122 (Hep3B/miR122) among the studied cell lines showed a critical improvement of HCV in cell culture engendering [22].

Upregulation of an instant objective of miR-122 and arginine carrier SLC7A1 belongs to solute transporter family 7 involve in prolonged increment of arginine level inside cell. Arginine served as substrate for enzyme nitric oxide synthetase and this enzyme levels were extended in HCC cells with silenced miR-122 expression inside cell by extended safety by use of a kinase inhibitor anti-cancer drug sorafenib [23]. The most notable mi-RNA reported in the development of human liver cancer is miR-122, which is thought to be a prospective marker linked to the damage of liver tissue [24]. Due to the existence of the HCV core, miR204-HPCAL1-lncRNAHOTTIP and miR-122-TGFBRAP1 pathways were reasonably connected with the progression of cancer. Mi-RNAs such as miR-122 and miR-204 over-expression may control the progression of HCC by down regulating the expression of TGFBRAP1 and HOTTIP [25]. The transcriptional studies reported implicated that hypoxia-inducible factor 1 α (HIF1 α) in the enlistment of miR-122 and recognized the oxygen-detecting prolyl hydroxylase space 1 (PHD1) as a miR-122 target. Further investigations showed that HIF1 α -subordinate acceptance of miR122 took part in a feed-forward pathway for liver security by means of the improvement of hepatic HIF responses through PHD1 repression [26]. Increased expression of GRHL2 in mice livers and liver of ALD patients inhibits the transcription of miR-122. Anti-miR-122 articulation reduced the extent of liver damage in mice after ethanol ingestion. By reducing HIF1 levels, miR-122 appears to protect the liver from damage brought on by ethanol. To diminish the harshness of ALD in patients, these cycles may be managed [27]. The capacity of miR-122 to advance separation of early stage and grown-up foundational microorganisms to hepatocytes *in vitro* suggests its expected outcome in dynamic hepatic separation program. As a key injury biomarker of liver, miR-122 has shown astonishing prospective for timely and susceptible *in situ* detection of DILI [28]. Glycyrrhetic acid has positive impact on DILI, but its administration is challenged by long-term and/or high dose effect [29]. By raising Sirt1 and activating the AMPK pathway, Long *et al.* found that miR-122 suppression protects hepatic cells from lipid metabolic trouble such as non-alcoholic fatty liver disease (NAFLD) and suppresses lipogenesis [30]. ADAM10 and c-Met are directly targeted by both mi-RNAs miR-122 and miR144. Upregulated expression of mi-RNAs miR-122 and miR-144 prompted diminished articulation of ADAM10 and c-Met in the UM cell lines and impaired cell expansion, movement, cell cycle, and flaking of c-Met ecto-space [31]. Sorafenib is commonly utilized in the treatment of HCC; however, the drug dose not process to be uniformly beneficial for all patients [32]. A major issue associated with sorafenib is the acquired resistance [33]. Being a conserved liver specific mi-RNA, miR-122 has been reported to play a key role in liver metabolism as well as homeostasis. miR-122 is looked on a potential molecule to act as biomarker for detecting/diagnostics of liver damage [28]. Long *et al.* reported miR-122 to be a potential biomarker and also a suitable drug target for NAFLD [30]. In the study, both *in vitro* (free fatty acid treated Hep G2 and Huh7) and *in vivo* (mice fed with high fat diet) were analyzed with observations of excess lipid production and triglyceride secretion in presence of miR-122 upregulation which downregulates Sirt1. MiR-122 inhibition resulted in suppressed lipogenesis and reduced excess lipid production. Fauda *et al.* reported utilization of combination of four miRNA named as miR-299, miR-200, miR-335, and miR-21) to identify HCC [34]. Most of the studies conducted pertaining to application of miR-122 as biomarker is confined to liver related disorders.

2.2. HCC Development by Oncogenic mi-RNAs

Oncogenic mi-RNAs are frequently expressed during different cancer cases such as breast, lung cancer, lymphoma, prostate cancer, melanoma, colorectal cancer, and liver cancer. OncomiRs are those dysregulated mi-RNAs that are upregulated in HCC and other cancer types. Many onco-miRNA types that are specific to their targets undergo specific mechanisms in tumor cell growth and invasion, whereby some are mentioned in Table 1. Specifically, miR-21 expression increased in cells, serum, and other body fluids of HCC patients [4]. miR-21 in HCC cases has oncogenic properties in most estimations [35]. Other miRNAs such as miR-21/-221/-451 suppress the activity of PTEN deleted on chromosome 10) tumor suppressor gene, a key control gene in cancer pathway [36,37].

2.3. HCC Development by Tumor Suppressor mi-RNAs

Several mi-RNAs expression is reported to be downregulated during HCC development that serves as tumor suppressor by targeting immune cells and proteins for activation and silence oncogenes by targeting specific mRNAs employed tumor cell growth and invasion [44]. The downregulated mi-RNAs may have the capacity to silence the oncogenic gene activity and modify oncogenic proteins by targeting their mRNA sequences. The mi-RNAs of the let-7 family were named as tumor-suppressors since they target RAS-associated protein families [45]. These mi-RNAs have control over genes related to hepatic fibrosis, lowering tumorigenicity. Details are given in Table 2.

Gramantieri *et al.* reported in their study that miR-122a is abundant in HCC cases among which nearly 60–70% was down-regulated, and hence confirms the hepatocarcinogenesis potential of the down-regulated miR-122a [48]. The expression of five miRNAs, that is, miR-671-3p, miR-486-3p, miR-328-3p, miR-378a-3p, and miR-378a-5p was identified in another study and it was reported to be in a significantly reduced state in HCC cells compared to normal cells

Table 1: Upregulated mi-RNAs functioning as oncogenic mi-RNAs for HCC

miRNA	Targets	Mechanism	References
miR-21	Inhibit Kruppel like factor 5. miR-21 upregulation activates HCC tumor cell invasion, development, and migration Target programmed cell death 4 factor	Cell growth, migration, and invasion	[38]
miR-25	Target on TNF-related apoptosis-inducing ligand	Apoptotic cell death	[39]
miR-96	Target and control SMAD signalling pathway	Promotes schistosomiasis-related hepatic fibrosis Increase collagen expression	[40]
miR-451	Target interleukin-6R-STAT3 pathway	Angiogenesis	[41]
miR-221	TRIAL resistance regulation and tumorigenicity enhancer through PTEN and tissue inhibitor of metalloproteinase	Cell death/apoptosis	[42,43]

HCC: Hepatocellular carcinoma

in HCC patients [46]. The expression level of onco-miRs has been correlated with tumorigenesis and/or tumor suppression. The miR-29 overexpression significantly reduces or inhibits tumorigenesis in an *in vivo* research study by promoting apoptosis and directly targeting antiapoptotic proteins, for example, BCL2 and MCL1 [49,50]. With the investigations and detailed research on dysregulated mi-RNAs in HCC cases, extracellular mi-RNAs can be applied as candidate biomarkers for HCC detection.

2.4. mi-RNAs as a Biomarker for HCC Associated with Various Diseases

The developmental stages of HCC in most patients are silent at early stages, that is, no initial symptoms have been shown by the patient. Many diagnostic techniques, imaging devices and conventional tumor markers such as α -fetoprotein, have been developed and adopted for diagnosing HCC and screening out tumor cells in the liver [51]. However, diagnosis with such conventional markers provides unsatisfactory results and increases the necessity for predictive biomarkers. The mi-RNAs possess potential application as an effective non-invasive biomarker for HCC prediction for their key role in fibrosis and liver regeneration [52]. The important mi-RNAs investigated till date to use as biomarkers for HCC and liver disease include miR-21, miR-121, miR-16, and miR-221 [53]. The HCC developmental stages are common in patients with liver

Table 2: Downregulated mi-RNAs functioning as a tumor suppressor.

miRNA	Targets	Mechanism	References
miR-122a	Modulate cyclin G1 expression	Control cell cycle	[46]
miR-486-3p	Target fibroblast growth factor receptor 4 and estimated glomerular filtration rate	Apoptosis the potent target for HCC treatment	[46]
miR-3622a-3p	Downregulate spalt-like transcription factor 4	Inhibit cancer cell proliferation and metastasis	[47]
miR-29b	Promote expression of tumor-suppressor genes	Repress DNA methyltransferase, Immune cell malignancy	[48]

HCC: Hepatocellular carcinoma

Table 3: Sensitivity and specificity of selected mi-RNAs as biomarkers in early-stage HCC [43,58,59].

mi-RNA	Sample	Sensitivity (%)	Specificity (%)
miR-21	Serum/plasma	84–89.5	71.2–92.0
miR-122	Serum	70.7–81.6	69.1–83.3
miR-29b	Serum	75.4	87.5–89.6
miR-15b	Serum	98.3	15.3
miR-215	Serum	78.7-80	91
miR-122a	Plasma	70.6	67.1
miR-223	Serum	80	76.5
miR-18a	Serum	86.1	75.0
miR-130b	Serum	87.7	81.4
miR-483-5p	Plasma	55.7	85.7

HCC: Hepatocellular carcinoma

cirrhosis, hepatitis B virus infection, etc. The mi-RNAs (upregulated/downregulated), circulating in the biological fluids of HCC patients, exist as apoptotic bodies or encapsulated exosomes or bound to serum proteins, lipids, and membranes [54]. The ability to circulate mi-RNAs, for example, miR-122 and miR-15b (upregulated miRNAs) in

the serum of HCC patients for earlier diagnosis of HCC in association with hepatitis B virus was reported [55]. A research study constructed a mi-RNA combination composed of has-miR-221 and has-miR-29c using step-wise logistic regression analysis and confirmed the combination as a promising tool for HCC diagnosis. Patients with

Table 4: Summary of clinical studies conducted to diagnose/analyze liver disorders through miRNA as biomarker.

mi-RNA	Clinical implication/Disease	Target cell/Molecule	Critical finding	References
miR-122	DILI	Glycyrrhetic acid	Clinical diagnosis and treatment of DILI	[29]
miR-122	mi-RNA as potential candidate to enhance hepatic ischemia tolerance	Murine modes of hepatic IR injury	Study depicted potential role of miR-122 in enhancing hepatic ischemia tolerance	[26]
miR-122	Identical of miR-122 to negatively affect expression of cytotoxic gene in λ AAV vector	miR-122 enriched Huh7 cell line	<ul style="list-style-type: none"> The study reported HEK293 miR-122 cells with 122T sequence to effectively attenuate cytotoxic transgene expression γ AAV vector possessing tcs-122T decreased hepatocellular carcinoma cell proliferation 	[60]
miR-122	Implication in NAFLD pathogenesis	HepG2 and Huh-7 cells	miR-122 inhibition protects liver cells from lipid metabolic disorders like NAFLD along with suppression of lipogenesis	[30]
miR-21		HepG2.2.15 and Huh7-1.3 cells	miR-21 higher expression in HCC group and miR-122 lower expression inhibited proliferation and invasion of HepG2.2.15 and Huh7-1.3 cells. Luciferase reporter gene along with RNA immunoprecipitation assay illustrated smad family 7 to be target gene of miR-21	[39]
miR-21	Diagnosis of HCC	PDK1/AKT	miRNA-21 could alter HSCs to CAFs by downregulation of its descendent target PTEN to activate signaling pathway PDK1/AKT. In extension, CAFs advanced cancer enhancement by angiogenesis through secretion of interleukins IL-6 and IL-8. Further crosstalk between HSCs and cancer cells explicate the molecular mechanism involved in HCC invasion	[61]
has- miR-122	Diagnosis of HCC		The study reported down regulation of has-miR-122 in HCC patients which further acts as biomarker for diagnosis of HCC in blood sample	[24]
Inc-RNAs, mRNA, Circ-RNAs	Diagnosis of HCC	Huh7 cell line	Over expressed miR-122, miR-204 was reported to be linked to inhibition of HCC progression through down regulation of TGFBRAP1 and HOTTIP	[25]
miR-122	miR-122 expression in ethanol induced liver disease	C57Bl/6 mice	GRHL2 inhibits miR-122 expression resulting in low level of miR-122	[27]
mi-RNA	Diagnosis of HCC	Epithelial-mesenchymal transition (EMT), HCC	mi-RNAs (122, 148a, 1246) raised in serum exosomes in HCC patients contrasted with liver cirrhosis (LC) and normal control (NC) individuals	[62]
mi-RNA	Development of inflammatory bowel diseases (IBDs)		Potential role of miR (miR -122, 196A2, 124A) polymorphism in clinical phenotype modulation among IBD like Crohn's disease and ulcerative colitis	[63]
miR-122	miR-122 regulation by free fatty acids and impact of miR-122 on triglyceride synthesis	Huh7 BNL-IME HEK293	Histological studies of different tissues from mice exhibited FFA to increase hepatic expression secretion of miR-122	[64]
miR-21	miR-21 dysregulates phosphatase and tengin homolog (PTEN) resulting inhibition of At R	HCC cells	miR-21 is potential therapeutic agent to overcome cellular resistance against sorafenib	[65]
miR-193a	HCC, Negative regulator of UPA	HCC cell lines	<ul style="list-style-type: none"> miR-193a transfected 00HA22T/VGH HCC cells exhibited anti proliferative potential miR-193a and sorafenib treatment in combination was reported to be complementary and resulted in further inhibition of cellular proliferation 	[32]
miR-122	Establish cell line for HCV propagation	HCV cc Huh7	New permissive cell line for HCV cc can be potential for research related to HCV and other therapeutic methods	[22]
miR-122	miR122 target gene expression in liver disease	HCC, HCV	miR-122 was recently shown to modulate miRNA and target gene articulation in the liver and result in the deficiency of HCV with negligible poison levels in a non-human primate	[66]

HCC: Hepatocellular carcinoma, HCV: Hepatitis C virus, DILI: Drug induced liver injury

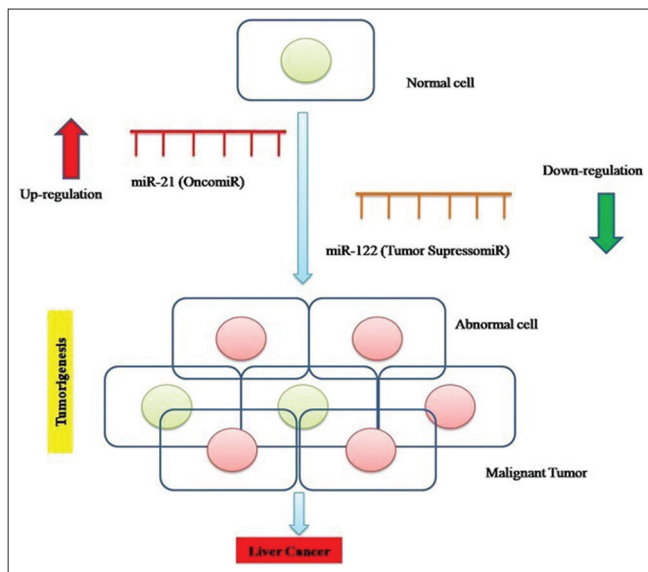


Figure 4: Illustration of liver cancer development mediated by miR-21 (oncomir) and miR-122 (tumor suppressor-miR).

HBV-associated HCC have significantly lower serum miR-101 expression compared to other cells in HBV-associated cirrhosis and hepatitis, suggesting that miR-101 has biomarker potential for detecting and identifying HBV-associated HCC [56]. The miR-122 proves to be a valuable diagnostic tool (as biomarker) in HCC caused by HCV. MiR-122, miR-885-5p, and miR-22 were all found to have diagnostic efficiency and accuracy for advanced HCC detection in patients with liver cirrhosis [57]. Table 3 summarizes specificity and selectivity of selected mi-RNAs.

3. THERAPEUTIC POTENTIAL OF MI-RNAS FOR HCC

Applications of mi-RNAs for the development of therapeutic targets for practical implementation are enticed by the regulation of numerous targets in cascades related to HCC pathway and enhancement of mi-RNA based cancer therapy. As already mentioned, the downregulated mi-RNAs act as tumor suppressors and activate tumor suppression proteins. The downregulated mi-RNAs can be restored in liver cancer patients as tumor treatment. In some cases, the upregulated mi-RNAs that act as onco-miRNAs were knocked down in HCC cases using the CRISPR/Cas9 system to silence the oncogenes activation [46]. Figure 4 illustrates an outline of down regulation of miR-122 and upregulation of miR-21 involved in tumor formation in liver cells. Table 4 summarizes research studied conducted to assess the potential role of different mi-RNA in HCC and related liver disorders.

4. FUTURE PROSPECTS AND CHALLENGES

Scientific studies on mi-RNA identification and continuous investigation of their biogenesis, regulatory mechanisms, and mi-RNA mediated gene modulation advances the utility of miRNAs for disease diagnosis. Mi-RNAs have been recognized as effective molecular tool for identification of candidate genes for various diseases and as biomarker are particularly under study for cancer diagnosis and development of treatment targets. Analysis of published literature on modulation of the mi-RNAs expression in various tumor affected cells, depict the potential role of mi-RNAs as biomarkers for liver diseases and HCC development. With specific reference to cancer biology,

several scientific studies established the sound role of mi-RNA to be utilized as biomarker for cancer diagnosis. Up-regulation and down-regulation of specific mi-RNA are directly related to increase/decrease in cancer cell proliferation through inflexing expression of genes metabolic pathways involved in cancer cell biology. Mi-RNAs effective approach in combination or with other markers and imaging techniques can be successful early diagnosis of liver cancer (HCC). Development of biological database along with other computer software programs specifically of mi-RNA study and analysis can confer valuable support analyzing diagnostic potential of different mi-RNA and their respective therapeutic potential. Gene level modification is another area which can be explored to optimize the application of mi-RNAs in healthcare and medicine. Development of process protocol and diagnostic kits is core potential target of mi-RNAs-based studies. Such objectives and goal-oriented research can change the perspective of diagnostics on global scale with mi-RNA's to be among key players.

5. CONCLUSION

The present review indicates that the biological aspects of mi-RNAs mainly miR-122 and miR-21 can be potentially employed as a biomarker and diagnostic tool for HCC diagnosis. There are existing challenges which needs to be addressed to explore complete potential of mi-RNA in the field of molecular diagnostics. Manipulation of pathways and biogenesis in immune cells could lead to new treatment methods for cancer, infectious illness, autoimmune diseases, and other immunological malignancies on a practical level. The combinations of mi-RNAs in place of targeting single mi-RNA could be utilized as effective early stage prognostic biomarker which provide high diagnostic accuracy and efficiency. Finally, it could be concluded that the molecular expression pattern of miR-122 and miR-21 provide molecular information for the development and progression of HCC.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

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