



## MicroRNA-122: A Key Factor in Chronic HCV infection

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### Abstract:

The 1989-discovered hepatitis virus type C (HCV) is a single-stranded RNA (of 9.6 kb) genome coding for about 3010 amino acid types. HCV infection is a significant health burden. Most often (55–85%), acute HCV infection progresses to chronic disease.

Little RNAs called micro-RNAs (miRNAs) are part of nearly every developmental or disease process, and in immunological and inflammatory responses as they control messenger RNA (mRNA) translation and mRNA stability. The aberrant regulation of miRNA is significantly linked to the occurrence and progression of numerous diseases.

MicroRNA-122 or “MiR-122”, is a miRNA exclusive to liver. According to several studies, genome stability, translation, and even replication of HCV have all been linked to it.

**Key words:** MiR-122, HCV, HCC.

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### Introduction:

Chronic HCV infection is a significant worldwide health issue, due to its high likelihood of progression to cirrhosis and ultimately HCC. According to a 2019 estimate from the WHO, there are 58 million persons globally with chronic HCV infection and new infections are about 1.5 million yearly. Moreover, 0.5% to 10% of HCV-related cirrhosis yearly results in HCC and about 290 000 people died from hepatitis C, predominantly from cirrhosis and HCC<sup>(1)</sup>.

The most typical form of hepatic cancer is HCC. Throughout the last ten years, the prevalence of HCC has nearly doubled, making it the third-most common reason for cancer fatalities worldwide<sup>(2)</sup>

Regarding Egypt, the primary cause for liver cirrhosis then HCC is HCV, and HCC is the leading cause of cancer death (about 32.35 % of all cancer fatalities)<sup>(3)</sup>.

A microRNA is single-stranded particles (nearly 22 nucleotides), plays a role in post-transcriptional

regulation of gene expression and RNA silencing via controlling mRNA stability and translation<sup>(4)</sup>.

MiRNAs are thought to control more than one-third of total human mRNAs<sup>(5)</sup> and numerous biological processes as cell division, growth, differentiation, metabolism, apoptosis, and signalling pathway transduction, are regulated by miRNAs<sup>(6)</sup>.

A variety of human disorders are linked to miRNAs' aberrant expression, especially cancer<sup>(7) (8)</sup>. So, they have been presented as crucial diagnostic and prognostic clinical biomarkers and as treatment options or possible therapeutic targets for many diseases and several cancer types and as novel cancer therapies<sup>(9) (10) (11)</sup>.

MiR-122 was discovered to have crucial role in HCV infection and in hepatocarcinogenesis occurrence, as patients with HCC showed differences in levels of miR-122 in the blood in comparison with healthy persons<sup>(12) (13)</sup>.

### MicroRNA-122 (MiR-122):

MiR-122 was initially identified in 2002 by cloning and sequencing mouse tissue-derived miRNAs. All vertebrates have been found to share a high degree of conservation for the miR-122 gene, which originates from a single genomic location on chromosome “18” and is controlled developmentally, its hepatic expression rises and over through the embryogenesis<sup>(14)(15)</sup>.

MiR-122 is essential for hepatic development, homeostasis, differentiation, and functions by regulating expression of a vast number of mRNAs related to many hepatic activities as well as by repressing nonhepatic genes. It also controls lipogenesis.

MiR-122 suppression through genetic and pharmacological techniques causes dysregulation of hepatocyte differentiation, iron homeostasis, and systemic and hepatic lipid metabolism<sup>(16)</sup>. Furthermore, research revealed that miR-122 is dysregulated during HCV infection,

### MiR-122 role in HCV infection:

#### MiR-122 controls the pathogenesis and viral tropism:

HCV is a virus that only infects hepatocytes, in part due to the fact that it depends on miR-122, extremely prevalent in hepatocytes<sup>(17)</sup>. The reasons for viral dependence on miR-122 are still not clear, but it is evident that miR-122 is necessary for

replication of HCV in cell culture and that its presence limits HCV replication to the liver which is beneficial for the virus<sup>(18)</sup>.

Additionally, it has been hypothesised that HCV reliance on miR-122 influences the pathogenesis of the virus by controlling virus tropism and maybe by affecting miR-122's normal function in cells<sup>(19)</sup>. MiR-122 controls the metabolism of lipids and cholesterol, the virus may benefit from miR-122's enhancement of cellular activities. In addition, reliance on miR-122 may successfully mute HCV allowing it to evade immune response, as the liver is considered an immune-privileged site<sup>(20)</sup>.

### MiR-122's role in promoting the HCV life cycle:

Unlike the commonly known function of miRNA suppression, miR-122 promotes HCV propagation via stabilizing its genome, as mutation of virus genome in binding site for miR-122 made HCV RNA less stable and inhibited replication<sup>(21)</sup>. Interestingly, there are Three main mechanisms for miR-122's ability to promote virus replication include:

1. Genome stabilization
  2. Stimulation of genome translation, by promoting the formation of canonical HCV IRES RNA structure.
  3. Directly enhancing genome multiplication<sup>(22)</sup>.
- (Figure 1)

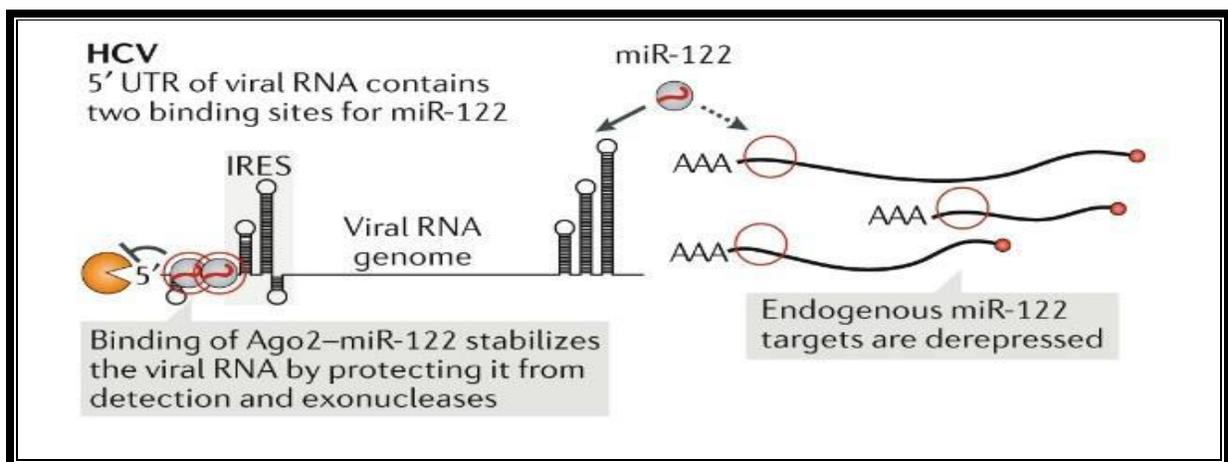


Figure (1): HCV viral mechanism modulating miR-122 function<sup>(31)</sup>.

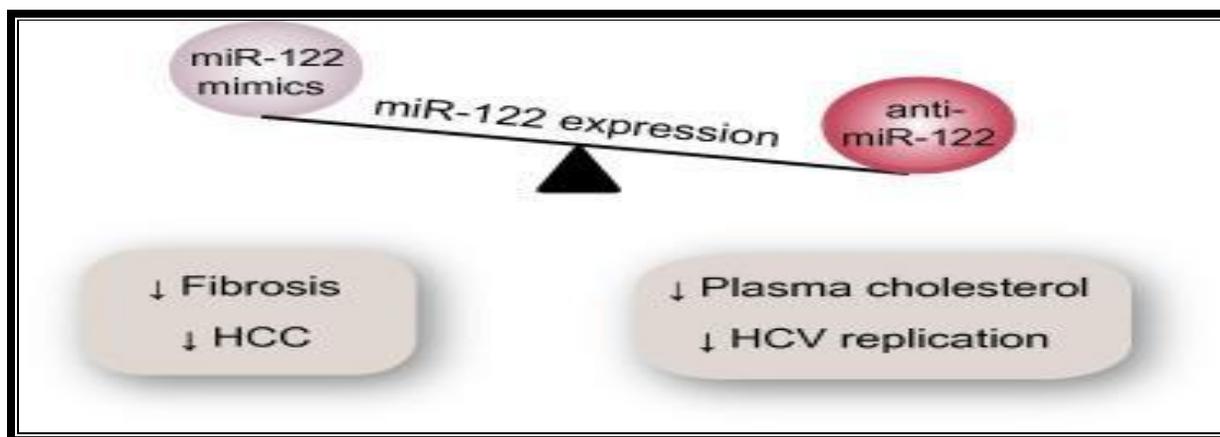
### MiR-122 as a potential HCV infection therapeutic target:

Because of its small size, specialized expression in the liver, and numerous roles, miR-122 has become an important target for both mimic and anti-mir therapies<sup>(23)</sup>.

Recent HCV therapy concepts consider miR-122 suppression as a possible therapeutic strategy since it promotes HCV replication through a variety of mechanisms, including increasing HCV

RNA translation and preventing HCV genome RNA breakdown<sup>(24)</sup>.

On one hand, antisense-mediated suppression of miR-122 (anti-miR-122) were shown to be clinically interesting therapeutic tool against hypercholesterolemia and for treating chronic HCV infection. On the other hand, miR-122 restoration (miR-122 mimics) has been proposed as a therapeutic strategy to avoid the emergence of HCC and hepatic fibrosis<sup>(25)</sup>. **(Figure2)**



**Figure (2): MiR-122-modulating therapies in liver disease<sup>(32)</sup>.**

Therefore, anti-HCV therapy already used it as a pharmacological target. As of 2017, “Miravirsen” was being developed by “Santaris Pharma” as a potential HCV therapy. It is a locked nucleic acid-based antisense oligonucleotide which suppresses miR-122. When taken with direct-acting antivirals, miravirsen also has an additive effect, making it potentially useful for patients not reacting to only direct-acting antivirals (DAAs)<sup>(26)</sup><sup>(27)</sup>.

A second anti-miRNA-122 medication underwent clinical testing recently, the anti-miRNA-122 oligonucleotide RG-101, which is conjugated to N-acetylgalactosamine. In Phase I trial, it dramatically decreased viral load when added to DAAs in all of the patients who were treated. To determine the effectiveness of combining RG-101 with DAAs to decrease treatment period, a phase II trial investigation was scheduled to begin<sup>(28)</sup>.

### Detection of chronic viral hepatitis using circulating miR-122 as a biomarker:

During cell death, exosomes and micro vesicles leak miRNAs into the bloodstream. These released

miRNAs are relatively non-invasive biomarkers to diagnose infections since they can be reliably measured in different body fluids including plasma, serum, cerebrospinal fluid (CSF), and urine<sup>(29)</sup>.

MiR-122 has reportedly been dysregulated in the blood in both HCV and HBV infection. Analysis revealed that its amount corresponded to infection stage and intensity and helped evaluate the treatment's effects. Moreover, it was established that in comparison to plasma, serum may offer a more favourable matrix for identifying miR-122 expression, proving its value in improving diagnostic accuracy of chronic viral hepatitis especially type C<sup>(30)</sup>.

### MiR-122 role in HCC:

#### MiR-122 as a diagnostic and prognostic biomarker for hepatitis C- related HCC:

AFP is employed as a common biomarker for early identification of HCC, however its sensitivity and specificity are inadequate. So, there is a pressing need to research and find novel non-invasive

diagnostic biomarkers that are feasible and accurate<sup>(31)</sup>.

MiRNAs are detectable from a variety of tissues, including the peripheral circulation, and exhibit differential expression between normal and malignant tissues. They also exhibit stable expression in serum and plasma (very stable). This makes them practical, non-invasive potential cancer screening biomarkers in clinics<sup>(32)</sup>. Many tumor-suppressing and oncogenic miRNAs in HCC are helpful for early diagnosis and for predicting prognosis<sup>(33)</sup>.

The verified miR-122 target genes ADAM10, IGF1R, SRF, Wnt1, cyclin G1, and ADAM17, have all been associated to inflammation, fibrosis, and cancer and were shown to be involved in angiogenesis, epithelial-mesenchymal transition, and hepatocarcinogenesis<sup>(34)</sup>. So, according to reports, the expression of miR-122 in HCC tissues, serum, and cell lines was dysregulated<sup>(35)</sup>.

MiR-122, a tumour suppressor, inhibits the growth of HCC via interacting with target HCC genes that regulate proliferation, differentiation, angiogenesis, cell migration, and apoptosis. For example, it targets cyclin G1 to inhibit growth of hepatocyte, and targets BCL-w and ADAM17 (implicated in metastasis) to increase hepatocyte apoptosis<sup>(36)</sup>.

Numerous clinical findings revealed that abnormally expressed miR-122 was related to a variety of clinical characteristics including tumour size, venous invasion, disease stage, and pathological differentiation. Bad prognosis and metastasis have been linked to reduced expression or loss of miR-122. Also, ALT level, albumin level, platelet count, and illness stage were all connected with the various miR-122 expression in HCC patients' circulation. While HCC's anti-tumorigenic capabilities were demonstrated by miR-122 overexpression in cell lines, Moreover, its overexpression cause HCC cells to be more sensitive to chemotherapeutic drugs as doxorubicin and sorafenib. This suggests a connection between HCC prognosis and miR-122 levels<sup>(37)</sup>.

### **Therapeutic application of miR-122 against HCC:**

Elevating miR-122 concentrations in HCC with or without anticancer drugs could be efficient HCC

therapeutic method because miR-122 is a liver-specific tumour suppressor miRNA. Several mechanistic studies have shown that miR-122, when artificially increased, inhibited growth, metastasis, and drug resistance of HCC tumour cells<sup>(38)</sup>.

Moreover, miR-122 modulates expression of genes responsible for multidrug resistance and the unfolded protein response, making HCC cells more susceptible to anticancer drugs like doxorubicin and sorafenib<sup>(40) (41)</sup>.

In fact, miR-122 gene transfer into cultured HCC cells causes arrest of cell-cycle and apoptosis<sup>(42)</sup>. Three-month-old miR-122 knockout mice received hydrodynamic injections of miR-122, which successfully inhibited hepatocarcinogenesis and tumour growth as evidenced by a decrease in tumour incidence and size<sup>(43)</sup>.

MiR-122 encapsulated in cationic lipid nanoparticles injected inside tumor significantly reduced HCC xenografts growth by about 50% and was associated with suppression of target gene and impaired angiogenesis<sup>(44)</sup>.

The proliferation of HCC cell lines is inhibited by a new MS2 bacteriophage virus-like particle-based miR-122 delivery method that is cross-linked with the HIV TAT peptide and can penetrate the cytomembrane<sup>(45)</sup>. Moreover, a graphene-P-gp loaded with miR-122-InP@ZnS quantum dots nanocomposites was created to induce apoptosis in HCC in order to overcome chemotherapeutic resistance<sup>(46)</sup>.

Nowadays, RNA molecules are being applied for the detection and treatment of numerous diseases<sup>(47)</sup>. A significant advancement in the diagnosis and prognosis of many liver diseases will come from the creation of novel technologies for direct detection in biological fluids and targeted miR-122 delivery to tumor cells. These technologies are urgently required for bringing basic research to the bedside<sup>(48)</sup>.

### **Conclusion:**

HCV infection results in the modulation of liver-specific miR-122 expression which was proved to enhance viral replication, hence it has a strong potential to serve as a novel noninvasive promising biomarker for liver injury for the diagnosis and

evaluation of patients with HCV. Moreover, miR-122 has been demonstrated to be involved in the process of hepatocarcinogenesis, acting as tumor-suppressing gene, thus may act as a novel diagnostic and prognostic biomarker for HCV-induced HCC.

According to clinical proof-of-concept trials, miR-122 inhibitors effectively lowered viral load in persistently infected HCV patients without exhibiting any signs of resistance. Furthermore, proliferation, metastasis, and treatment resistance of HCC tumor cells were also evidently inhibited by artificially upregulating the miR-122 gene, according to numerous mechanistic studies.

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