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RESEARCH ARTICLE

REVIEW ARTICLE: HCV IMPACTON THE PATTERN OF CIRC-RNAEXPRESSION IN HCV RELATED HCC

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Abstract

Circular RNAs (circRNAs) are a class of non-coding RNAs with a circular, covalent structure, which are highly conserved, stable unique molecules found in eukaryotic cells. The most notable character of circRNAs is their high stability in biological systems which is a key factor in their potential for use in a variety of RNA-focused medical applications. Despite the fact that circRNAs have a wide range of biological roles and regulatory functions, their circular structure and sequence overlap with their linear mRNA counterparts make it difficult to research them in depth. Furthermore, little is known about their function in viral infections and in immune responses.Since circRNAs have been found to be involved in a number of viral infections (including hepatitis B virus infection and human papilloma virus infection), their significance in viral infections is being more recognized in the last years.In this review, we aimed to provide a broad basis and overview on the biogenesis, significance and regulatory roles of circRNAs in the context of viral hepatitis C virus (HCV) infection, and HCV related hepatocellular carcinoma. Owing to the fact that the chronic HCV infection is the leading cause of HCC in the western countries especially Egypt. HCV can act directly on cell signaling pathways to promote HCC by inhibiting tumor suppressor genes or by causing activation of signaling pathways that up-regulate growth and division. Because viruses frequently manipulate cellular pathways to control viral gene expression, cellular and viral circRNA landscapes in virus-infected cells are altered. Studying the contribution of circRNAs in these pathways may give valuable information in the pathogenesis of HCC caused by HCV or in finding more reliable therapeutic approaches.

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Introduction:-

Circular RNAs

Are a group of non‑ codingRNAs widely found in eukaryotic cells, with a circular andcovalently‑ bonded structure**(Hansen et al., 2013; Salzman et al., 2013)**. In the twenty-first century, with the development of RNA sequencing technologies and bioinformatics, the abundance and diversity of circRNAs was identified, and the dynamic expression patterns of circRNAs were revealed in various developmental stages and physiological conditions **(Memczak et al., 2013)**. Regulatory non-coding RNAs (ncRNAs) have emerged as important participants in diverse normal and pathology- associated biological functions.

CircRNAs were first discovered in 1976 on viroid particles. Then, circular structures were identified by electron microscopy in the cytoplasm of eukaryotic cells, notably in HeLa cells **(Sanger et al., 1976)**. Later were found to be an endogenous RNA splicing product in eukaryotes in 1979 as well **(Hsu and Coca-Prados, 1979)**.However no specific biological function was reported for circRNAs at that time **(Zaphiropoulos, 1996)**. Afterwards there was a subsequent gap in the field until 2013. At this time, **Memczak et al** and **Hansen et al** reported on regulatory roles of circRNAs, actually, they showed evidence for miR-binding capacity of the circular sequences **(Hansen et al., 2013; Memczak et al., 2013)**.

Fundamentally, circRNAs are covalently closed by a phosphodiester linkage between downstream donor and upstream acceptor RNA splice domains. Previously was considered as splicing errors**(Hsu and Coca-Prados, 1979)**, circRNAs are then accepted as functional RNA molecules **(Salzman et al., 2013)**. They display tissue- and cell-specific expression patterns and are encoded from thousands of genes. Emerging evidence demonstrates that circRNAs are involved in biological processes contributing to the onset and progression of cancer **(Li et al., 2020)**.

The delayed emergence of the role of circRNAs as regulators include: 1) absence of a poly(A) sequence which excludes circRNAs from commonly used mRNA purification protocols, 2) assumption that the sequencing reads across the back-splicing junctions were artefact, and 3) inability of early RT-PCR procedures to distinguish between linear and circular RNAs **(Memczak et al., 2013)**. Since 2013, several new bioinformatics algorithms based on back-splice junction overlapping reads recognition made it possible to efficiently detect denovocircRNAs and to differentiate them from their linear counterparts **(Ma et al., 2019)**. Accordingly, the number of circRNA libraries drastically increased and repository databases (e.g. circBase) were established to annotate circRNAs**(Glažar et al., 2014)**. It is now accepted that circRNAs are the most abundant RNA isoforms, originating from thousands of human genes and that their expression is conserved in eukaryotes **(Louis et al., 2021)**.

Biogenesis

A growing body of research demonstrates that circRNA is produced during mRNA processing before splicing. CircRNAs are generated by a non-canonical splicing mechanism, called back-splicing. They result from canonical splice sites and depend on canonical splicing machinery, which is usually inefficient to generate linear RNAs **(Memczak et al., 2013; Salzman et al., 2013)**. Through a general mechanism; downstream 5’ splice donor site from a pre-RNA transcript reacts with an upstream 3’ splice acceptor site, which results in the 3’ extremity of a downstream exon joining to the 5’ extremity of an upstream exon. Therefore, the circularisation junction is formed of 2 mis-ordered exons (according to their genomic location) as shown in **Figure (1),** this is for the formation of exoniccircRNA (ecircRNAs)**.** According to the structure, circRNAs are categorised into four groups: ecircRNAs, circular intronic RNAs (ciRNAs), exon-intron circRNAs (EIciRNAs), and tRNAintronic circular RNAs (tricRNAs), which are made up of tRNA introns. More than 80% of the discovered circRNAs are ecircRNAs, and the majority are found throughout the cytoplasm **(Jeck et al., 2013)**. The significant nuclear localization of ciRNAs and EIciRNAs, however, suggests that they might control gene transcription **(Zhang et al., 2019)**.

Several mechanisms can be involved in the canonical splicing can be discussed **(Tang et al., 2021)**:

1- **Circularization is brought on by RNA-binding protein (RBP):** for connecting related intronic sequences. RBPs like Quaking genes are known as trans-acting factors could cause a closer proximity between the 3′ and 5′ ends of the circularised exons and ease splicing **(Errichelli et al., 2017)**.
2- **Back-splicing may be improved by pairing with a complementary inverted sequence:** The characteristic intronic sequence permits the splice donor to be close to the splice acceptor, hence facilitating nucleophilic attack and cleavage **(Ivanov et al., 2015)**.
3- **Lariat-induced circularization driven by spliceosomes:** The change in 5′ splice locations supports the hypothesis that exon circularization is spliceosome-dependent. Spliceosomes are gathered at the back-splicing

site to help join the 5′ donor and 3′ acceptor sites. As a result, internal splicing takes place in the lariat, resulting in the release of ecircRNAs or EIciRNAs**(Starke et al., 2015)**.

4- **tricRNA splicing:** Pre-tRNA must be split into two pieces by tRNA splicing enzymes in order for tricRNA to form 3′-5′ phosphodiester bonds required for circularization. RNase P and RNase Z, respectively, split the leader and trailer. The cleavage of pre-tRNA produces two exon halves and the intron, each have 5′OH and 2′,3′cyclic phosphate at the cut locations.

CircRNA functions

Many different functions of circRNAs have been defined including actions as:

1- Sponges of endogenous miRs, numerous circRNA harbour miRNA response elements. Therefore, by sponging miRNA, circRNA act as competitive endogenous RNA, preventing miRNA post-transcriptionally binding to their mRNA targets **(Memczak et al., 2013; Hansen et al., 2013)**.

2- Templates for translation of short proteins: Although circRNAs are non-coding RNAs, the presence of an internal ribosome entry site (IRES) upstream of the circRNA start codon can allow the 40S subunit of eukaryotic ribosomes to bind and initiate translation **(Wang and Wang, 2015)**.

3- Sponges of proteins, circRNA display specific protein binding motifs offering them the capability to sequester RNA binding proteins (RBP), regulate their activity and influence their localisation **(Hentze and Preiss, 2013)**.

4- Transcription regulation: exonic-introniccircRNA regulate transcription by interacting with small nuclear ribonucleoproteins (snRNPs) and promoting the transcription of their parental genes**(Li et al., 2015b)**.

5- Protein scaffolding: Proteins can be recruited by circRNA, to facilitate enzymatic reactions there are growing number of studies indicate that circRNAs act as scaffolds in the formation of functional complexes **(Du et al., 2016; Du et al., 2020)**

Sponging miRs is the most commonly described function, and was also the first reported effect of circRNAs on gene expression **(Hansen et al., 2013; Memczak et al., 2013)**. It is well-established that miRs play important roles in cellular processes that lead to malignant transformation. Since they partially hybridize to mRNA targets and inhibit translation through action of the RNA-Induced Silencing Complex (RISC). By sequestering miRs, circRNAs cause de-repression of target mRNA **(Rupaimoole et al., 2016)**. If the target mRNA encodes an oncogenic protein, then circRNA sponging leads to overexpression of the potentially transforming protein **Figure (1).**

Stability of CircRNAs:

Circularity confers stability and resistance to exonucleolytic destruction for 3′-5′-linked circRNAs, raising the question of whether circRNAs could be useful as stable diagnostic and therapeutic targets or agents. Moreover, due to the abundancy of exonucleases in biological fluids, circRNAs show high stability in biological systems, consequently some of the reported circRNAs having 10-fold higher expression levels compared to related linear RNAs Therefore, it is not surprising that circRNA could be ideal biomarker for disease **(Patop et al., 2018)**. Furthermore, extracellular association of circRNAs with 40-100nm small exosomes or extracellular larger microvesicles has suggested that circRNAs can spread from cell-to-cell **(Maass et al., 2017)**.These are the most notable traits and a key criteria for why circRNAs could be used for a variety of RNA based medical applications in contrary to their linear counterpart. Although circRNAs have variety of biological activities and regulatory functions, studying them in depth is difficult due to their circular form and sequence overlap with the linear mRNA **(Awan et al., 2021)**.

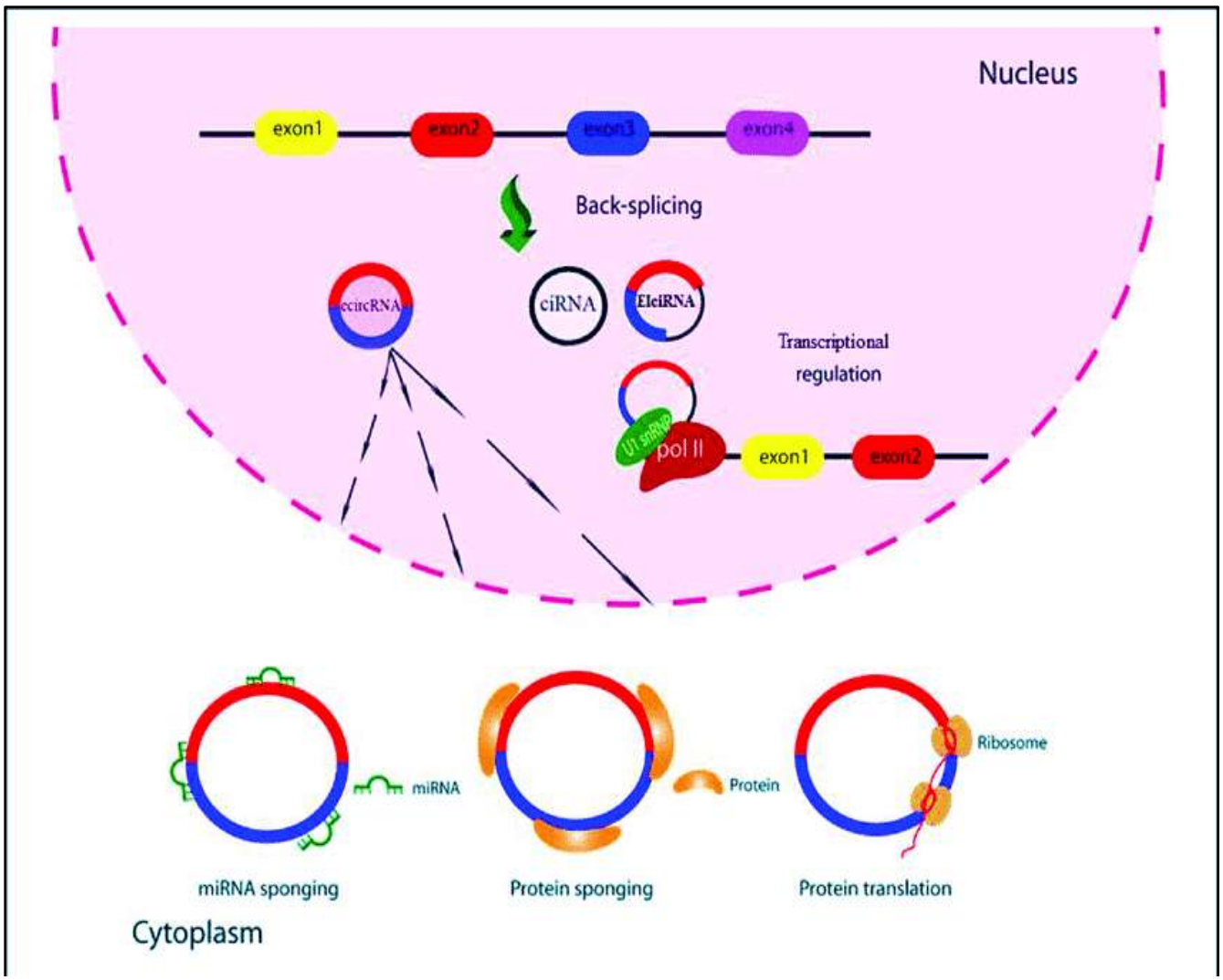

Figure (1):- Schematic diagram of circRNA synthesis and functions adopted from (Nahand et al., 2020).

CircRNAs might function as miRNA sponges by competing for the binding of miRNA sequences, lessening the impact of miRNA-mediated regulation of gene expression. CircRNAs might function as protein sponges. Some circRNAs might control the expression of proteins by sequestering mRNA translation start sites. CircRNAs might be translated to create functional proteins.

Detection methods for circRNA

CircRNA detection is currently primarily accomplished via RNA-Seq and microarrays. The main use of RNA-seq is the identification of new circRNAs. The only direct proof that circRNAs may be found is at head-to-tail junctions**(Li et al., 2019)**. RNaseR is typically used to process total RNA in order to demonstrate the stability of circRNAs by removing linear RNAs while leaving circRNAs intact. The fluorescently tagged complements for circRNA was then hybridised to microarray probes, which stained the head-to-tail junctions.For confirmation and more precise quantitation of circRNAs, standard PCR-based techniques such as reverse transcription quantitative PCR (RT-qPCR), droplet digital PCR (dd PCR), and northern blot are thought to be the most frequently used In addition, Fluorescence in-situ hybridization is utilised to pinpoint the amount and distribution of circRNA for further investigation of related functions **(Wang et al., 2022)**. Currently, a variety of online databases, including circBase, CIRCpedia, CircInteractome, and Circ2Traits, have been built to study the data, regulatory network, and the function of circRNA in illnesses and other physiological processes. The function of circRNAs will be better understood as circRNA identification methods and databases continue to advance**(Wang et al., 2022)**.

CircRNA in Hepatocellular carcinoma (HCC)

HCC is a highly heterogeneous malignancy derived from complicated genetic and epigenetic alterations. The challenges opposing hepatologists worldwide are based on how to screen HCC patients at the earlier stage and thereby perform timely curative procedures, such as radical primary resection, ablation or liver transplantation. Therefore, exploring the molecular mechanism and identifying valuable markers of HCC are extremely important**(Dhanasekaran et al., 2016)**.

Liver cancer is rated as the 6th most common cancer worldwide, and the 4th leading cause of cancer-related death in the world according to the International Agency for Research on Cancer. GLOBOCAN 2018.**(Llovet et al., 2021)**. The highest incidence and mortality of HCC are observed in East Asia and Africa, even though HCC incidence are increasing in different parts of Europe **(McGlynn et al., 2015)**.

Data from the recent literature suggest that circRNAs are relevant molecules involved in regulatory networks leading to HCC onset and progression **(Fu et al., 2018; Ely et al., 2021; Louis et al., 2021; Wang et al., 2022)**. The impact of circRNAs in some hepatocarcinogenic pathways was well described as cell proliferation, apoptosis, migration and invasion. Several circRNAs are widely described in literature for their implications in pathogenesis, diagnosis or therapy in liver cancer **(Louis et al., 2021; Ely et al., 2021; Hao et al., 2019; Wang et al., 2022)**.

Prevention of HCC could be achievedbycontrollingits risk factors. HBV and HCV remain the major risk factors, and it is estimated that there are 250 million chronic carriers of HBV who are at high risk for HCC. Prophylactic vaccination against HBV has been implemented to avert HCC**(Hu et al., 2019)**.HCV infection is the other important risk factor for HCC. Approximately 140 million people are infected with HCV and at risk for HCC but there is no effective vaccine against this virus.**(Ringelhan et al., 2018)**.

HCV related HCC

Chronic HCV infection is the leading cause of HCC in the western countries. Risk of HCC in HCV-infected patients increases by 15- to 20-fold, with annual incidence of HCC being estimated at 1% to 4% in cirrhotic patients **(El-Serag, 2012; Abd-Elsalam et al., 2018)**.Hepatitis C virus (HCV) is the major cause of HCC in the majority of Asia and South Africa USA, Europe, Japan and South America and our country Egypt **(Yang et al., 2010; Abd-Elsalam et al., 2018; Llovet et al., 2021)**. It is estimated that 2.5% of the world's population (177.5 million) are infected with HCV **(Petruzziello, 2018)**.

HCV induced carcinogenesis

Is a stepwise process spanning over 20 to 40 years. HCV carcinogenesis is mediated by viral-induced factors and host-induced immunologic response Studies have shown that the HCV core protein may drive lipogenesis and impair oxidative stress metabolism. HCV viral proteins can act directly on cell signaling pathways to promote HCC by inhibiting tumor suppressor genes and cell cycle check points or by causing activation of signaling pathways that up-regulate growth and division. **(Axley et al., 2018)**.Specific tumor suppressor genes inhibited by HCV core protein include retinoblastoma protein and p53 tumor suppressor. The loss of p53 and retinoblastoma is synergistic, leading to a greater degree of carcinogenesis. HCV nonstructural protein genes also promote fibrosis and the development of HCC through inducing transforming growth factor-beta and activating hepatic stellate cells **(Lemon and McGivern, 2012; Axley et al., 2018)**.

CircRNAs Interplay with viral infection

CircRNAs are involved in the regulation of viral infections, such as hepatitis B virus (HBV) and human herpes virus(HHV) infections **(Awan et al., 2021)**. In addition, circRNAs have been proposed as biomarkers to distinguish viral from non-viral pneumonia **(Zhao et al., 2019)**. The crucial role of circRNAs in viral infections is associated with controlling miRNA levels and regulating innate immune responses**(Awan et al., 2021)**.

Because viruses frequently manipulate cellular pathways to control viral gene expression, various researchers have studied whether the cellular and viral circRNA landscapes in virus-infected cells are altered. Indeed, infected cells by Epstein-Barr virus (EBV) and Kaposi's sarcoma virus (KSHV) not only alter the cellular setting of circRNAs, but also display circRNAs from viral pre-RNA transcripts **(Tagawa et al., 2018)**. Moreover some findings suggested that circRNAs encoded by viruses might play an important role in the host-virus interaction by regulating viral and host gene expression together with modulation of the host's innate immunity, **Huang et al., 2019,** identified an EBV-encoded circRNA (ebv-circRPMS1), and showed that this circRNA is localized both in

cytoplasm and nuclei of host cells , and have potential functions in regulation of host and viral gene expression and thus playing important roles in the host-virus interaction.**(Huang et al., 2019)**.

CircRNAs have been shown to influence different aspects of viral infections, including virus replication and pathogenesis. Moreover, both DNA and RNA viruses produce regulatory circRNAs in addition to host-derived circRNAs that are modified and/or used during infection **(Cadena and Hur, 2017)**. The exact machinery for how both host and virally encoded circRNAs coordinate to benefit or retard virus replication is still not well proved. Further experimental investigations and testing are needed to define how virally encoded circRNAs contribute to immune escape and how viruses affect host circRNAs also, whether circRNAs act as virulence factors or no. **(Awan et al., 2021)**. **Figure (2)**

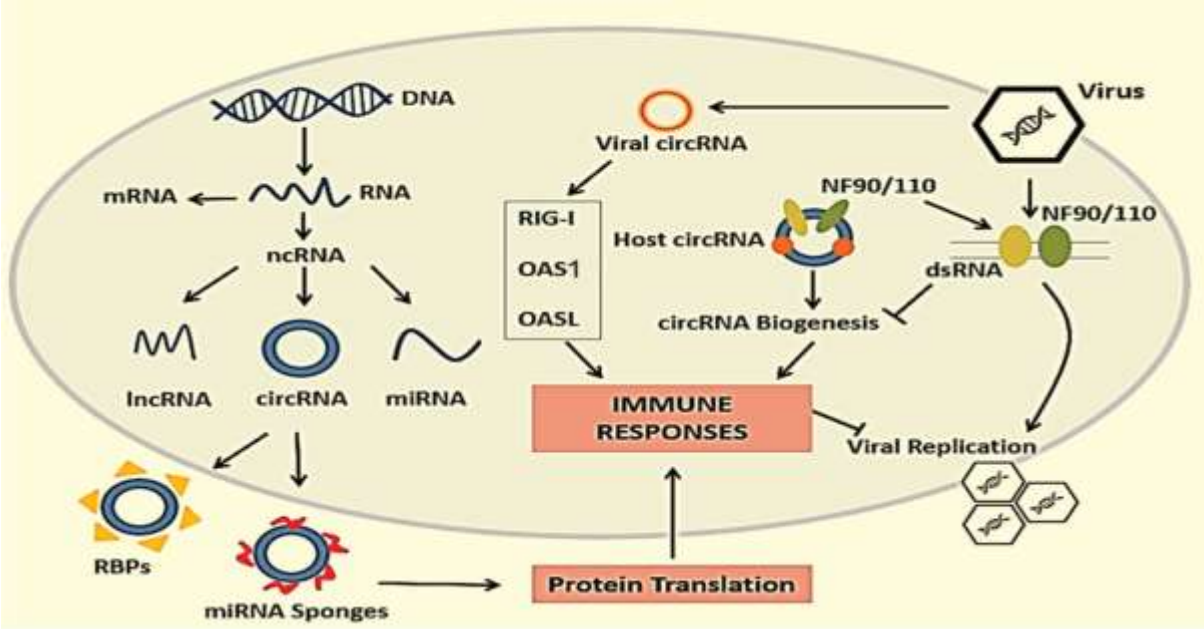

Figure (2):- Schematic representation of the functional significance of circRNAs in the antiviral immune responses. (Awan et al., 2021).

Nuclear factor (NF) complexes 90/110 (NF90/NF110) promote circRNA production in the nucleus by associating with intronic RNA pairs and interacting with mature circRNAs in the cytoplasm through binding sites. Viral circRNAs induces host immune response (OAS) 2′-5′-oligoadenylate synthetase family, which include OAS1, OAS2, OAS3, and OAS-like (OASL) protein. OASL was identified to be strongly induced following viral infection through engaging the RNA sensor RIG-I a

CircRNAs in hepatitis C virus (HCV) infection

Inside infected host cells, viruses have shown to deregulate various cellular signalling pathways involved in antiviral functions to neutralise innate immune responses and to aid in viral gene amplification.

Non-coding RNAs (ncRNAs) in host cells, including microRNAs (miRNAs) have been reported to play major roles in HCV replication, miR-122 favours HCV genome replication by binding to the non-coding region of the HCV viral RNA promoting its stability and preventing its degradation **(Thibault et al., 2015)**. Nevertheless, limited data is available regarding the regulatory role of circRNAs during HCV infection.

Recently, **Chen et al.,** examined the circRNA profile in uninfected and HCV infected liver cells. The authors performed RNA sequencing and reported that one of the upregulated circRNA, circPSD3, displayed a very pronounced pro-viral effect and promoted replication of HCV genotype 1a and 2 **(Chen et al., 2020)**. In another study, **Jost et al.,** examined the effect of artificial circRNA (harbouring miR-122 binding sites) on HCV model system which requires cellular miR-122 for its life cycle. They found that in a full-length infectious HCV cell culture system, the artificial circRNA inhibited viral protein production by sequestering cellular miR-122. The

findings of the study revealed that viral protein production was strongly suppressed with comparable efficiency to Miravirsen, the first antimiR-drug against HCV infection **(Jost et al., 2018)**.

Concerning the significance of circRNAs in HCVinfection,there was an unusual finding; where**Chen et al.,** noted when examining the circular RNA landscape in uninfected and HCV infected liver cells, that all differentially produced circRNAs in HCV infected cells are exhibiting infection-induced abundance changes that cannot be explained by corresponding abundance changes of their linear counterparts. An example was seen in their search where circTIAL1 whose abundance increases after infection, whereas its linear transcript is down-regulated **(Chen et al., 2020)**. This was also observed in our previous work on circ-ITCH when studied in HCV infected HCC patients, where we found an unexpected upregulation of circ-ITCH in the plasma of HCC patients compared to healthy controls, in contrary its linear counterpart that was down expressed **(El Sharkawi et al., 2022)**.

This can be partiallyexplained by: the tendency of HCV infection to alter splicing site preferences on certain identified genes and thereby increases the likelihood for back splicing for all identified genes, also; the outstanding stability of circRNAs compared to linear counterparts can explain this differential expression. Additionally HCV may alter the back splicing frequency as well. Although testing these hypotheses would require a more detailed analysis of the regulatory mechanisms of back splicing before and after the viral infection **(Chen et al., 2020)**.

CircRNA in viral hepatitis related HCC

CircRNAs in HBV related HCC is well studied in literature **(Schweitzer et al., 2015)**. Many studies addressed the expression levels of host circRNAs affected by HBV infections leading to HCC.**Zhou et al. (2018)** studiedcircRNAs controlling HBV infection and progression to HCC, they found thathsa_ circ_0005389, hsa_circ_0000038were both significantly upregulated, whilethe hsa_circ_0000650/mir-6873 and hsa_circ_0000650lmir-210-5pwere downregulated. They found that circRNAs areinvolved in HBV replication, as well as in the liver inflammationand fibrosis caused by HBV infection.**(Zhou et al., 2018b)**.Another study showed that the expression levelof circ_00004812 has been found to be increased inHBV‐ infected cells, whichhave been reported to participate in the oncogenesis processinduced by HBV infection**(Zhang and Wang, 2020; Yang et al., 2020)**.Moreover, theexpression levels of several circRNAs have been described to be alteredin the plasma of HBV‐ related patients with cancer **(Yu et al., 2020)**, hsa_circ_0027089 is another circRNA upregulated in the plasma of patientssuffering from HBV‐ related HCC**(Zhu et al., 2020)**.**Liao et al.(2021**) published a recent review where they collected most molecular biology and therapeutic strategies for circRNAs in HBV-related HCC**(Liao et al., 2021)**.

Unfortunately, studies addressing circRNAs in HCV related HCC isvery scanty.Even by taking into consideration that HCV is the leading etiology of HCC in the USA, Europe, Japan and South America, Egypt**(Yang et al., 2010; Abd-Elsalam et al., 2018; Llovet et al., 2021)**.

In 2018 **Jost et al.,**studied the effect of artificial circRNA (harbouring miR-122 binding sites) on HCV model system which requires cellular miR-122 for its life cycle. They found that in HCV cell culture system, the artificial circRNA inhibited viral protein production by sequestering cellular miR-122 and though proposed circRNA as a therapeutic agent for sequesterin mir-122**(Jost et al., 2018)**. Afterwards, **Chen et al.,**study examined the circular RNA scene in uninfected and HCV Infected liver cells, they showed that distinct classes of circRNAswere up-regulated or down-regulated in infected cells but they didn't relate their finding to HCV related HCC**(Chen et al., 2020)**.

Our research team, worked in this field but in clinical aspect, as we planned to study the differential expression of circ-ITCH and its linear counterpart in the plasma of Egyptian HCC patients (HCV positive) comparing to healthy subjects, and to investigate their correlations with liver function parameters , to determine the possible diagnostic ability of plasma circ-ITCH as a stable non-invasive marker compared to its linear counterpart**(El Sharkawi et al., 2022)**.

Circ-ITCH was suggested to play an anti-tumor role by controlling miRNA activity, it was stated in literature to be down regulated in many cancers **(Li et al., 2015a; Wan et al., 2016; Luo et al., 2018)**. The results of our study came in contrast to those findings, as circ-ITCH was up regulated in the plasma of HCC patients particularly the non-metastatic patients while its linear counterpart lin ITCH had an opposite behavior, where it showed significantly lower expression levels in metastatic and non-metastatic HCC groups than control group.

We there tried to explain the conflicting results between our results and what has been published in literature by some factors, the type of specimen which was plasma while others were focused on the tumor tissue specimen, or cultured cell lines. We assumed that circ-ITCH might be decreased in liver tissues, as was stated in literature, and is pooled from liver tissues to blood due to hepatocyte injury caused by HCV infection, since circulating RNAs may be passively released from broken cells**(Turchinovich et al., 2011)**. Also, Due to the fact that circRNA are more stable than linear RNA in body fluids and though higher expression levels were seen in circRNAs exclusively**(Maass et al., 2017)**.

But , the morecompelling explanation that we concluded that the etiology of HCC, which is related to HCV is the main factor , rather than HBV, targeted in several studies such as **(Zhou et al., 2018a; Cui et al., 2018a).** Wepostulated that HCV may have a different effect on circ-ITCH, where HCV core protein may be one of these substrates that can undergo ubiquitination and proteasomal degradation by ITCH protein **(Shirakura et al., 2007)**. ITCH protein expression is affectedby the upregulation of circ-ITCH specifically. Consequently we suggested that HCV core protein may affect circ-ITCH expression in plasma samples of HCV related HCC patients.**(El Sharkawi et al., 2022)**

This was also confirmed by **Chen et al.,**they examined the circular RNA scene in uninfected and HCV Infected liver cells, they found differentially expressed circRNAs in infected and un infected cells**(Chen et al., 2020)**.

Irrespective of the involvedmechanisms, we were unable to directly predict which of the altered circRNAsin HCV infection are infact functional, or which of them its expression was altered by mechanistic effect from HCV.

Chen et al.,concluded that; it is uncertain that all identified circRNAs with altered abundances in HCV-infected cells have distinct functions, since circRNAsmay display RNA sequences or structural motifs that are recognizedby interacting RNAs or by protein molecules that modulate viral pathogenesis. But certainly,they found many circRNAs displaying small imperfect duplexes that bind to protein kinase R (PKR) (which is identified as a cellular protector against viral infection and is a major regulator of central cellular processes including mRNA translation, transcriptional control, regulation of apoptosis, and cell proliferation**(Gal-Ben-Ari et al., 2019)**) and thereby prevent PKR from being activated, suggesting that circRNAsmay display bulk functionsaffectinig host cells. Thus, enhanced abundances of specific circRNAs in virus-infectedcells may have bulk functions in infected cells, or in neighboring cells after vesicle-mediateddelivery of circRNAs**(Chen et al., 2020)**.

These findings illuminate the light on the critical importance of circRNAs in HCV infection and that altered expression ofcircRNAs is expected to become an important molecular tool for the early diagnosis of HCV‐ related HCC, which can result in an increasedpatient long‐ term survival, reducing the mortality rate andimproving prognosis. However, limited data is available regarding the regulatoryrole of circRNAs during HCV infection, and so further studies examining circRNAs in HCV related HCC is required to figure out the role of circRNAs in the pathogenesis and then using them as important therapeutic agent.

HCV infectionhardly result in sterilizing immunity, although direct acting antivirals (DAAs) have been so effective, some people now believe that HCV is curable and that further study of the virus has little clinical value .Subjects who have been treated with DAAs still run the risk of having the virus again. In light of this, it is highly recommendedthat research must give gross attention towards the global eradication of HCV and its risk causing to HCC.

The rapid development of high‐ throughput sequencing andbioinformatics in recent years has allowed for the detection of highly expressed circRNAsin virus infection‐ related diseases.The fact that circRNAs are abundant, ubiquitous, stable,conserved across species and tissue‐ cell specific indicatesa diagnostic potential greater than that of linear RNA. CircRNAs have the intrinsic latency to be perfect biomarkers since they can bear out properties including, adequate variation in normal and abnormal physiological conditions, stable in body fluids as saliva, blood, gastric fluid, exosomesand tissues, cell to cell distinct and specific expression,increased stability and high sensitivity**(Cui et al., 2018b)**. In the same way, the regulatory role of circRNAs in various human diseaseshighlights their role as therapeutic agents and/or targets.

CircRNA-based molecules broaden the range of drug targets and important genes that aren't normally regarded viable to conventional drug targets. With the advancement of molecular technology and the development of reliable sequencing procedures, these unusual pharmacological targets can now be easily identified.

Jost et al.,has reported that artificial circRNAs can be engineered asmiRNA sponges to sequester disease-related miRNAs.Theengineered circRNA construct was able to inhibit miR-122and the HCV translation in a cell culture model was repressedsignificantly**(Jost et al., 2018)**.These discoveries gavea promising imagination to the emerging circRNA basedtherapeutics.

In conclusion:-

We tried to focus on the critical importance of circRNAs in HCV infection being an important molecular tool for the early diagnosis of HCV‐related HCC, which can result in an increased patient survival rate and improving prognosis. More comprehensive studies examining circRNAspattern of expression in HCV related HCC are required to figure out the role of circRNAs in the pathogenesis and then using them as important therapeutic agent.

Future suggestions of these studies towards clinical applications in terms of therapeutics will require an intense and coordinated effort across many heads. The more we know about circRNA expression patterns in diseases and the processes by which they work, the greater our chances of improving diagnosis, prognosis, and developing better treatment agents.

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Authors' contributions:

This review, was a conclusion after our team's study published before **(El Sharkawi et al., 2022)**. Where Walaa M.N had the major contributor in writing the manuscript. Dr.Sahar A.A, Dr. Mamdouh E.S, made substantial amendments to the manuscript and Dr.Fathia Zmade significant contributions to conception, design, and provided final approval for the study to be published. All authors read and approved the final manuscript.

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