Chapter (I)

BIOCHEMISTRY OF microRNA

**1**- **Historical Review**

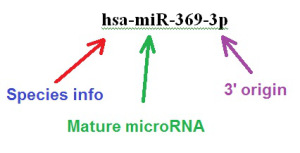
microRNAs (miRNAs) are small, non-coding ribonucleic acid (RNA) molecules that regulate gene expression at the level of translation. Although the first miRNA was discovered twenty years ago, only in the last few years, the breadth and diversity of this gene class had been uncovered ***(Pasquinelli and Ruvkun, 2002).***

***In 1993,*** ***Victor Ambros, and collegues*** discovered the first miRNA during a study of the gene *lin-14*in *Caenorhabditis*elegans (*C. elegans*) development. They found that the *lin-4* gene encoded a 61-nucleotide (nt) precursor matured to a 22-nucleotide RNA. This short RNA contained sequences partially complementary to multiple sequences in the *lin-14* messenger RNA (mRNA) and regulated LIN-14 protein abundance. In 2000, a second RNA was characterized: *let-7*, which repressed *lin-41*, *lin-14*, *lin-28*, *lin-42*, and *daf-12* expression during developmental stage transitions in *C. elegans* ***(Reinhart et al., 2000).***

*Lin-4* and *let-7* did not encode proteins but rather RNAs which were extremely small (22nt) and were derived from hairpin structured RNA precursors ***(Wightman et al., 1993).*** It was soon realized that similar sequences, first termed microRNAs by ***Ambros*** in ***(2001)***, were wide spread in the genomes of eukaryotes ***(Lau et al., 2001)***.Since this time, a thousand of microRNAs has been cloned and characterized from a diverse range of organisms including arthropods, nematodes, vertebrates, plants and viruses ***(Lewis et al., 2005).***

## 2- Nomenclature

The name for a miRNA consists of the prefix “mir” followed by a dash and a number. The number indicates order of naming. For example, mir-123 was named and discovered prior to mir-456. The uncapitalized (r) in "mir-" refers to immature miRNA, while a capitalized (R) in "miR-" refers to the mature form. miRNAs with nearly identical sequences except for one or two nucleotides are annotated with an additional lower case letter. For example, miR-123a is closely related to miR-123b. Precurser-miRNAs (pre-miRNA) that lead to 100% identical mature miRNAs but that are located at different places in the genome are indicated with an additional dash-number suffix. For example, the pre-miRNAs mir-194-1 and mir-194-2 lead to an identical mature miRNA (miR-194) but are located in different regions of the genome **(fig. 1)** ***(Ambros et al., 2003)***.



***Fig. (1): Nomenclature of miRNA (***[***http://www.mirbase.org***](http://www.mirbase.org)***).***

Species of origin is designated with a three-letter prefix, e.g., *Homo sapien* -miR-123 is a human miRNA (hsa-miR-123) and *Ovis aries* -miR-123 is a sheep miRNA (oar-miR-123). Other common prefixes include 'v' for viral miRNA encoded by a viral genome and’d’ for *Drosophila* miRNA (for example d-mir-281in Drosophila. When two mature microRNAs originate from opposite arms of the same pre-miRNA, they are denoted with a -3p (3′ arm) or -5p (5′ arm) suffix. In the past, this distinction was also made with’s’ (sense) and 'as' (antisense). When relative expression levels are known, an asterisk following the name indicates a miRNA expressed at low levels relative to the miRNA in the opposite arm of a hairpin, for example, miR-123 and miR-123\* would share a pre-miRNA hairpin, but more miR-123 would be found in the cell ***(Griffiths-Jones et al, 2006).***

**3**- **Structure and biosynthesis of miRNA**

miRNA is a 21-24 nucleotides small RNA that is the processed product of a non-coding RNA gene. The mature miRNA is produced from a hairpin precursor after several steps ***(Ganguli et al., 2010).*** Most miRNA genes are found in intergenic regions or in anti-sense orientation to genes and contain their own miRNA gene promoter and regulatory units ***(Lau et al., 2001)***.

### (A) Transcription:

miRNA genes are usually transcribed by [RNA polymerase II](http://en.wikipedia.org/wiki/RNA_polymerase_II). The polymerase often binds to a promoter found near the deoxy ribonucleic acid (DNA) sequence encoding what will become the hairpin loop of the pre-miRNA. The resulting transcript is [capped](http://en.wikipedia.org/wiki/5%27_cap) with a specially modified nucleotide at the 5` end, [polyadenylated](http://en.wikipedia.org/wiki/Polyadenylation) with multiple [adenosines](http://en.wikipedia.org/wiki/Adenosine_monophosphate) (a poly (A) tail) and [spliced](http://en.wikipedia.org/wiki/RNA_splicing).The product is called primary miRNA (pri-miRNA). It may be hundreds or thousands of nucleotides in length and contain one or more miRNA [stem-loops](http://en.wikipedia.org/wiki/Stem-loop) **(fig. 2 page 6)** ***(Lee et al., 2004).***

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***Fig (2): miRNA biogenesis*** ***(Biggar and Storey, 2011).***

As much as 40% of miRNA genes may lie in the exons. These are usually regulated together with their host genes. The DNA template is not the final word on mature miRNA production. Human miRNAs show RNA editing, the site-specific modification of RNA sequences to yield products different from those encoded by their DNA. This increases the diversity of miRNA action beyond that implicated from the genome alone ***(Rodriguez et al., 2004)***.

### (B) Nuclear processing

A single pri-miRNA may contain from one to six miRNA precursors. These hairpin loop structures are composed of about 70 nucleotides each. Each hairpin is flanked by sequences necessary for efficient processing. The double-stranded RNA structure of the hairpins in a pri-miRNA is recognized by a nuclear protein known as [DiGeorge Syndrome Critical Region 8](http://en.wikipedia.org/wiki/Pasha_%28protein%29) (DGCR8) or "Pasha" in invertebrates, named for its association with [DiGeorge Syndrome](http://en.wikipedia.org/wiki/DiGeorge_Syndrome). DGCR8 associates with the enzyme [Drosha](http://en.wikipedia.org/wiki/Drosha), a protein that cuts RNA, to form the "microprocessor" complex. In this complex, DGCR8 orients the catalytic RNase III domain of Drosha to liberate hairpins from pri-miRNAs by cleaving RNA about eleven nucleotides from the hairpin base (two helical RNA turns into the stem). The resulting hairpin, known as pre-miRNA, has two-nucleotide overhang at its 3` end, it has 3` hydroxyl and 5` phosphate groups ***(Gregory et al., 2006)***.

Pre-miRNAs that are [spliced](http://en.wikipedia.org/wiki/RNA_splicing) directly out of [introns](http://en.wikipedia.org/wiki/Intron), bypassing the microprocessor complex, are known as "[mirtrons](http://en.wikipedia.org/wiki/Mirtrons)." originally thought to exist only in *Drosophila* and *C. elegans*. Mirtrons have now been found in mammals ***(Berezikov et al., 2007)***.

About 16% of pri-miRNAs may be altered through nuclear [RNA editing](http://en.wikipedia.org/wiki/RNA_editing), Most commonly by enzymes known as adenosine deaminases acting on RNA (ADARs) that catalyze transition of adenosine to inosine (A to I) ***(Winter et al., 2009).***

### (C) Nuclear export

Pre-miRNA hairpins are exported from the nucleus in a process involving the nucleocytoplasmic shuttle [Exportin-5](http://en.wikipedia.org/wiki/XPO5). This protein recognizes a two-nucleotide overhang left by the RNase III enzyme Drosha at the 3` end of the pre-miRNA hairpin. Exportin-5-mediated transport to the cytoplasm is energy-dependent, using guanisine triphosphate (GTP) bound to ras-related nuclear protein ([Ran](http://en.wikipedia.org/wiki/Ran_%28biology%29)) ***(Murchison and Hannon, 2004).***

### (D) Cytoplasmic processing

In the cytoplasm, the RNase III enzyme Dicer cleaves the pre-miRNA hairpin. This endoribonuclease interacts with the 3` end of the hairpin and cuts away the loop joining the 3` and 5` arms, yielding an imperfect miRNA:miRNA\* duplex about 22 nucleotides in length.  Although either strand of the duplex may potentially act as a functional miRNA, only one strand is usually incorporated into the RNA-induced silencing complex (RISC) where the miRNA and its mRNA target interact ***(Lund and Dahlberg, 2006).***

**4**- **Mechanism of action**

1. **The RNA-induced silencing complex**

The mature miRNA is a part of an active RISC containing Dicer and many associated proteins ***(Rana, 2007)***. RISC is also known as a microRNA ribonucleoprotein complex (miRNP) ***(Schwarz and Zamore, 2002)***.

Dicer processing of the pre-miRNA is thought to be coupled with unwinding of the duplex. Only one strand is incorporated into the miRISC, selection based on its thermodynamic instability and weaker base pairing relative to the other (The strand whose 5`end is less tightly paired to its complement is selected to enter into the RNAi-induced silencing complex) strand ***(Krol et al., 2004)***. The other strand, called the passenger strand due to its lower levels in the steady state, is denoted with an asterisk (\*) and is normally degraded. In some cases, both strands of the duplex are viable and become functional miRNA that target different mRNA populations ***(Okamura et al., 2008).***

Members of the argonaute (Ago) protein family are central to RISC function. Argonautes are needed for miRNA-induced silencing and contain two conserved RNA binding domains: P-element induced wimpy testis in [*Drosophila*](http://en.wikipedia.org/wiki/Drosophila) (PIWI), a domain that structurally resembles ribonuclease-H and functions to interact with the 5` end of the guide strand. Another domain is PIWI argonaute and zwille domain (PAZ) that can bind the single stranded 3` end of the mature miRNA and orient it for interaction with a target mRNA. Some argonautes, for example human Ago2, cleave target transcripts directly; argonautes may also recruit additional proteins to achieve translational repression (***Pratt and MacRae, 2009)***. The human genome encodes eight argonaute proteins divided by sequence similarities into two families: AGO (with four members present in all mammalian cells and called E1, F2, C and hAgo in humans) and PIWI (found in the germ line and hematopoietic stem cells) (***Schwarz and Zamore, 2002).***

Additional RISC components include [TRBP](http://en.wikipedia.org/wiki/TARBP2) [human immunodeficiency virus transactivating response RNA (TAR) binding protein] ***(MacRae et al., 2008)***, protein activator of the interferon induced protein kinase (PACT), fragile X mental retardation protein (FMRP), and Tudor staphylococcal nuclease-domain-containing protein (Tudor-SN)***(Mourelatos et al., 2002).***

**(B) microRNAs and short interfering RNAs (siRNAs)**

The process of RNA interference (RNAi) can be moderated by either [siRNA](http://biotech.about.com/od/glossary/g/siRNA.htm)  or [miRNA](http://biotech.about.com/od/glossary/g/Mi-rna.htm), but there are differences between the two. Both of them are processed inside the cell by the Dicerand incorporated into RISC**(fig.3 page: 11) *(***[***Tomari et al., 2004***](http://genesdev.cshlp.org/content/20/5/515.long#ref-87)***).***

|  |  |  |
| --- | --- | --- |
| **Differences** | **microRNAs** | **siRNAs** |
| Configuration | Single stranded. | Double stranded. |
| Source | miRNA comes from endogenous source (made inside the cell). | siRNA is considered exogenous that is taken up by cells or enters via [vectors](http://biotech.about.com/od/proteintechnology/g/Vectors.htm) like viruses. |
| Biogenesis | Expressed by genes whose purpose is to make miRNAs, but they regulate genes (mRNAs) other than the ones that expressed them. | Regulate the same genes that  express them. |
| Complementarity to target mRNA | Pairing is imperfect, so miRNA can inhibit [translation](http://biotech.about.com/od/proteintechnology/g/Translation.htm) of many different mRNA sequences. | siRNA binds perfectly to its mRNA target, so siRNAs can knock down specific genes. |
| Action | Cleave mRNA or inhibit its translation | Cleave mRNA |

***Table (1): Differences between miRNAs and siRNAs (mack, 2007).***

### http://www.microrna.ic.cz/obr/image003.png

### *Fig. (3): Differences between miRNAs and siRNAs* *(He and Hanon, 2004).*

### (C) Mode of Silencing

Gene silencing may occur via either mRNA degradation or preventing mRNA from being translated. It has been demonstrated that if there is complete complementation between the miRNA and target mRNA sequence, Ago2 can cleave the mRNA and lead to direct mRNA degradation. Yet, if there is not complete complementation, the silencing is achieved by preventing translation ***(Lim et al., 2005).***

First manner in which miRNAs and siRNAs control post-transcriptional gene expression is by directing endonuclease cleavage of the target mRNA. Such endonuclease cleavage, referred to as “Slicer” activity ***(***[***Allen et al., 2005***](http://genesdev.cshlp.org/content/20/5/515.long#ref-1)***)***. The products of miRNA mediated cleavage appear to be degraded by the same enzymes that degrade bulk cellular mRNA ***(***[***Parker and Song, 2004***](http://genesdev.cshlp.org/content/20/5/515.long#ref-70)***).***

A second way that miRNAs silence mRNAs is by interfering with their translation. For example, *lin-4* miRNA reduces the amount of LIN-14 protein, without reducing the amount of the *lin-14* mRNA ***(***[***Wightman et al., 1993***](http://genesdev.cshlp.org/content/20/5/515.long#ref-89)***)***. Silencing by a miRNA is observed with either no change in the mRNA level, or with a significantly decrease in mRNA levels than that is observed for protein ***(***[***Cimmino et al., 2005***](http://genesdev.cshlp.org/content/20/5/515.long#ref-15)***)***.

## (D) How do miRNAs repress translation?

miRNA-mediated repression can reduce translation initiation. Alterations in the translation initiation process can make a mRNA resistant to miRNA-induced translation repression. For example, tethering of the translation initiation factors (eIF-4E or eIF-4G) to a mRNA makes it resistant to miRNA-induced repression ***(***[***Pillai et al., 2005***](http://genesdev.cshlp.org/content/20/5/515.long#ref-72)***).***

**5*-* Functions of miRNA in normal conditions**

**(A) Role of miRNA in embryogenesis**

The role of miRNAs is investigated in development by examining mice that lack the essential miRNA-processing enzyme Dicer and therefore theoretically lack all miRNA functions. Targeted knockout of Dicer in mice causes embryonic lethality, suggesting an essential role for miRNAs in development, moreover, Dicer-deficient embryonic stem cells (ES) are defective in differentiation both in vitro and in vivo and do not form the three germ layers normally found in embryonic stem cells ***(Bernstein et al., 2003).***

MiR-1–1 and miR-1–2 are abundant in the developing heart. Enrichment of miR-1–1 is initially observed in the atrial precursors before becoming ubiquitous in the heart, whereas miR-1–2 enhancer is specific for the ventricles in the developing heart; therefore, miR-1–1 and miR-1–2 might have chamber-specific effects in vivo ***(Zhao et al., 2005)****.* miR-206, a close homolog of miR-1, is only expressed in skeletal muscle. As in the case of cardiac muscle, miR-1 and miR-206 promote the differentiation of skeletal myoblasts in culture. miR-133a has the opposite effect, inhibiting differentiation and promoting myoblast proliferation ***(Chen et al., 2006)*** .

miRNAs can be important regulators of neuronal structure and plasticity. In mammals, miR-134 is specifically expressed in the rat dendritic spine in hippocampal neurons. It binds to 3`untranslated region (UTR) of lim domain containing protein kinase 1 (Limk1). This miRNA represses local Limk1 translation resulting in inhibition of dendritic spine growth. Stimuli such as brain-derived neurotrophic factor can relieve this suppression ***(Schratt et al., 2006).***

Most somatic cells in *C. elegans* and *Drosophila* are post-mitotic and lack adult somatic stem cells; by contrast, germ line cells maintain pluripotential ability to renew themselves indefinitely. Dicer and Drosha loss-of-function mutants in *C. elegans* exhibited poor proliferation,. indicating a role for miRNAs in germ line stem cells ***(Knight and Bass, 2001).***

**(B) miRNAs and regulation of cellular differentiation**

MiRNAs may play a role in maturation of human adipocytes. Understanding the molecular events involved in adipocyte differentiation is of interest for development of therapeutics for metabolic diseases such as obesity and diabetes ***(Rangwala and Lazar, 2000)****.* One miRNA, miR-143, was identified which normally promotes adipocyte differentiation ***(Esau et al., 2004).***

**(C) miRNAs and cellular proliferation and apoptosis**

The involvement of miRNAs in cell death regulation was first reported 3 years ago when the first two miRNAs; miR-14 and *bantam*, were shown to regulate apoptosis in *Drosophila*. miR-14 inhibits apoptosis and increase proliferation . Heterozygous loss of function mutation for a miR- 14 (50% reduction) showed an enhancement of the small-eye phenotype. Conversely, overexpression of the miR-14 gene could suppress the small-eye phenotype in a dose dependent manner. Even the late-onset retinal cell death caused by expression of the initiator caspase *Drosophila* Nedd2- like caspase (Dronc) could be suppressed by miR-14 ***(Xu et al., 2003).***

*Bantam* is a gene causing overgrowth phenotype of *Drosophila* through increasing cell number. It turned out to encode a miRNA. *Bantam* could be expressed at all developmental stages, although higher in proliferating cells. Clones having two wild-type copies of bantam were three folds bigger in size. Thus, *bantam* can autonomously promote cell proliferation ***(Brennecke et al., 2003).***

Overexpression of the oncogenes, myelocytoma virus (Myc) or E2F in mammalian cells induces simultaneously both proliferation and apoptosis ***(Pelengaris et al., 2002).*** In contrast, stimulation of proliferation by *bantam* is accompanied by inhibition of apoptosis through reduction of E2F ***(Brennecke et al., 2003).***

**(D) Role of miRNAs in metabolism**

miRNAs function in all organs directly related to the metabolism of glucose, namely, the pancreatic islet, liver, skeletal muscle, adipose tissue and brain ***(Poy et al., 2007).***

miR-122 is liver specific miRNA with approximately 50,000 copies per cell. One study using specific cholesterol-conjugated antisense miRNA inhibitors, termed antagomirs, addressed the abolishment of miR-122 in vivo and its effect on glucose and lipid metabolism was studied. Complete degradation of miR-122 was observed after 3 days. While plasma cholesterol levels were reduced 4 days following treatment, no changes were observed in plasma glucose or triglyceride levels ***(Krutzfeldt et al., 2005).***

A second study implemented a similar antisense oligonucleotides technology (ASO) against miR-122 in mice and confirmed the effect on plasma cholesterol butmeasured a significant decrease in plasma triglycerides as well ***(Esau et al., 2006).*** Furthermore, primary hepatocytes isolated from the ASO-miR-122-treated mice displayed decreased hepatic fatty acid synthesis and sterol synthesis in addition to increased fatty acid oxidation. These changes were accompanied by an increase in the levels of AMP-activated kinase, an established inhibitor of fatty acid and cholesterol synthesis. Target genes of mir-122 mediating the effects on cholesterol are not known ***(Teleman et al., 2006).***

While typically not classified as a metabolic tissue, the brain and peripheral nervous system are widely known to influence glucose homostasis by signaling to the liver, muscle, pancreatic islets and gastrointestinal tract ***(Woods et al., 2006).***

The brain is an insulin-sensitive tissue that in turn affects the release of a variety of secreted signals from peripheral metabolic tissues. In addition to being implicated in learning and memory, cAMP response element–binding protein (CREB) is known to function in glucose homostasis as well ***(Mayr and Montminy, 2001).*** One study provides evidence for CREB to regulate miR-132 ***(Impey et al., 1998).*** Furthermore, several miRNAs were identified during the cloning from the mouse pancreatic β -cell line in the brain, suggesting an overlap in the function of these particular sequences ***(Poy et al., 2004)***, for example, miR-9, a miRNA shown to influence the release of insulin, was previously believed to be expressed exclusively in neuronal tissues ***(Krichevsky et al., 2003).***

**(E) Role of miRNAs in insulin secretion**

In 2004, studies showed that miR-375 could regulate glucose-induced insulin secretion, through independent role of glucose metabolism or intracellular Ca2+ signaling but correlated with a direct effect on insulin exocytosis. Myotrophin (Mtpn) was predicted to be a target of miR-375. ***(Poy et al., 2004)***.

In 2009, miR-375 wasknocked out in mice (375KO), 375KO mice are hyperglycemic, exhibit decrease in  cell mass, increased total pancreatic -cell numbers, fasting & fed plasma glucagon levels, and increased gluconeogenesis and hepatic glucose output. Bioinformatics analysis of transcript data from 375KO islets revealed that miR-375 regulates cellular growth and proliferation. These data provide evidence that miR-375 is essential for normal glucose homeostasis, -cell and -cell turnover, and adaptive -cell expansion in response to increasing insulin demand in insulin resistance.Thus, miR-375 is a regulator of insulin secretion and may a novel pharmacological target for the treatment of diabetes***(Poy et al., 2009)*.**

**(F) miRNA acting in signal transduction pathways**

Both strength and direction of signaling networks are dictated, in part, by protein abundance, this characteristic makes signaling networks ideal candidates for miRNA-mediated regulation ***(Cui et al., 2006).***

Mitogen-activated protein kinase (MAPK) pathways are key in regulating stress responses and transducing extracellular signals to cytoplasmic and nuclear effectors.The MAPK superfamily consists of three main protein kinase families: extracellular signal regulated kinase (ERK), c-Jun N-terminal kinase and p38 ***(Cowan and Storey, 2003).***

Each of these kinases plays a major role in the regulation of gene expression and intracellular adenosine triohosphate (ATP) metabolism ***(Storey and Storey, 2004).***

MAPK cascades detect, amplify and integrate diverse external signals to generate responses such as changes in protein activity, gene expression and may provide a conduit for a rapid response in stress-responsive miRNA expression ***(Terasawa et al., 2009).***

In response to DNA damage, signaling networks activate the p53. It is a tumor suppressor protein, which leads to the transcription and translation of effectors capable of triggering both cell cycle arrest and apoptosis ***(Inui et al., 2010).***

It was shown that miR-125b targets the 3` UTR of p53 and is essential for complete repression of its translation incidentally. miR-125b is itself down regulated after DNA damage, resulting in a higher p53 activation threshold and is sufficient to allow the translation of p53 and induce p53-dependent apoptosis and cell cycle arrest, two pathways critical to the typical DNA damage response ***(Zhang et al., 2009).***

**6- miRNA and pathological conditions**

**(A) miRNA and cancer**

In 2005, analysis of expression of 217 mammalian miRNAs in 334 samples including multiple human cancers was done. The resulting miRNA expression proﬁles were informative, reﬂecting the developmental lineage and differentiation state of the tumors. Poorly differentiated tumors could be classiﬁed much more accurately using the expression proﬁles of the 217 miRNAs than with mRNA expression proﬁles. Interestingly, the authors observed a general downregulation of most miRNAs in tumors when compared to normal tissues. However, particular miRNAs were speciﬁcally upregulated depending on the tumor type. The authors speculated that global miRNA expression might reﬂect the state of cellular differentiation. Therefore, the general downregulation of miRNAs could indicate that those cancer cells regained a more ‘stem-cell like character’ ***(Jovanovic and Hengartner, 2006).***

mir-17cluster is of particular interest. It is frequently amplified in B-cell lymphomas ***(He et al., 2005).*** Overexpression of the mir-17cluster was found to accelerate tumor development in a mouse B-cell lymphoma model ***(O’Donnell et al., 2005).***

**(B) miRNA and neurological diseases**

Spinal muscular atrophy (SMA), a progressive neurodegenerative disease, is caused by deletion or loss of function mutations in the survival of motor neuron protein (SMN) ***(Dostie et al., 2003)***. SMN is a component of the miRNP complex that performs the effector functions of the miRNA pathway ***(Mourelatos et al., 2002)***

Inactivation of the gene coding for fragile X mental retardation protein (FMRP), which is also associated with miRNP complex formation, cause fragile X syndrome. These studies indicate that disruptions in the miRNP machinery and hence miRNA activity can lead to disease states ***(Jin et al., 2004).***

The interaction of mir-189 ***(Abelson et al., 2005)*** in addition to mir-134 regulation in hippocampal neurons controls spine development and possibly also contributes to synaptic development, maturation and plasticity thus dysregulation of mir-134 could potentially lead to complications in these processes ***(Schratt et al., 2006).***

**(C) miRNA and viral infection**

Host mir-32 expression restricts infection of the primate foamy virus 1(PFV-1). Inhibition of mir-32 leads to doubling of the PFV-1 proliferation rates in host cells. PFV-1 encodes the Tas protein, which is known to be a suppressor of RNA silencing thereby removing the growth limitation inflicted by mir-32 by disrupting the silencing machinery ***(Lecellier et al., 2005).***

Many viruses encode similar suppressors of RNA silencing – for example, the Tat protein from human immunodeficiency virus 1 (HIV-1) ***(Bennasser et al., 2005)*** and the B2 protein from Nodamura virus ***(Sullivan and Ganem, 2005).***

miRNAs represent an efficient mechanism for viruses to use to manipulate host machinery, as they require less space on the viral genome than alternative protein products. Viral miRNAs can target both viral and host mRNAs for repression. Twelve miRNAs from the Kaposi sarcoma-associated herpes virus (KSHV) genome expressed in cells led to the down regulation of a number of genes including thrombospondin 1 (THBS-1), which is a known tumor suppressor and antiangiogenic factor ***(Samols et al., 2007)***

The simian virus 40 (SV40) encodes a miRNA that is perfectly complementary to transcripts coding viral T antigens, leading to their degradation. This destruction of viral T antigens aids the virus in evading immune detection by the host ***(Sullivan et al., 2005).*** The hepatitis C virus (HCV) enhances replication via a novel interaction of abundantly expressed mir-122 with the 5` UTR of the viral genome ***(Jopling et al., 2005)***.

Interferons (IFNs) are key molecules involved in eliciting the antiviral response once an infection has been detected ***(Cullen, 2006)***. IFN-b has recently been implicated in the activation of several miRNAs in mammals that have antiviral properties against hepatitis C virus (HCV) and treatment leads to reduced mir-122 expression ***(Pedersen et al., 2007)***, which limits HCV replication ***(Jopling et al., 2005)***. These studies identify a number of different miRNAs that could be therapeutically targeted to hinder viral infection, aid host detection of infection, and prevent viral manipulation of host machinery ***(Hennessy and O’Driscoll, 2008).***

Chapter (II)

Chronic hepatitis C

**1**- **Historical review**

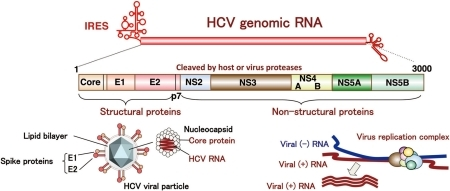
In the mid of ***(1970),*** [***Harvey J. Alter***](http://en.wikipedia.org/wiki/Harvey_J._Alter)***,*** chief of the Infectious Disease Section in the Department of Transfusion Medicine at the [National Institutes of Health](http://en.wikipedia.org/wiki/National_Institutes_of_Health), and his research team demonstrated how most post transfusion hepatitis cases were not due to [hepatitis A](http://en.wikipedia.org/wiki/Hepatitis_A) or [B](http://en.wikipedia.org/wiki/Hepatitis_B) viruses. Despite this discovery, international research efforts to identify the virus, initially called non-A, non-B hepatitis (NANBH), failed for the next decade. In (***1987), Michael Houghton, Qui-Lim Choo, and George Kuo*** at [Chiron Corporation](http://en.wikipedia.org/wiki/Chiron_Corporation), collaborating with ***Dan Bradley***  from centers for disease control ([CDC](http://en.wikipedia.org/wiki/Centers_for_Disease_Control_and_Prevention)) used a novel [molecular cloning](http://en.wikipedia.org/wiki/Molecular_cloning) approach to identify the unknown organism and develop a diagnostic test **(*Houghton, 2009).***

***Alter*** In ***(1988)*** confirmed the virus by verifying its presence in a panel of NANBH specimens. In April 1989, the discovery of the virus, renamed hepatitis C virus (HCV), was published in two articles in the journal Science ***(Choo et al., 1989).***

**2**- **Virology**

**(A) Structure of HCV**

(HCV) is a small (55-65 [nm](http://en.wikipedia.org/wiki/Nanometre) in size), enveloped, [positive-sense](http://en.wikipedia.org/wiki/Sense_%28molecular_biology%29#Positive-sense), single-stranded [RNA](http://en.wikipedia.org/wiki/RNA) [virus](http://en.wikipedia.org/wiki/Virus) of the family [Flaviviridae](http://en.wikipedia.org/wiki/Flaviviridae). HCV particle consists of a core of genetic material, surrounded by an [icosahedral](http://en.wikipedia.org/wiki/Truncated_icosahedron) protective shell of [protein](http://en.wikipedia.org/wiki/Protein), and further encased in a lipid envelope of cellular origin (derived from host cell during replication). Two viral envelope [glycoproteins](http://en.wikipedia.org/wiki/Glycoprotein) spikes (E1 and E2) are embedded in the lipid envelope **(fig.4)** ***(Op De Beeck and Dubuisson, 2003)***.



***Fig. (4) : Structure and genome organization of hepatitis c virus (***[***Moriishi and***](http://www.ncbi.nlm.nih.gov/pubmed?term=Moriishi%20K%5BAuthor%5D&cauthor=true&cauthor_uid=22347882) [***Matsuura***](http://www.ncbi.nlm.nih.gov/pubmed?term=Matsuura%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=22347882) ***, 2012).***

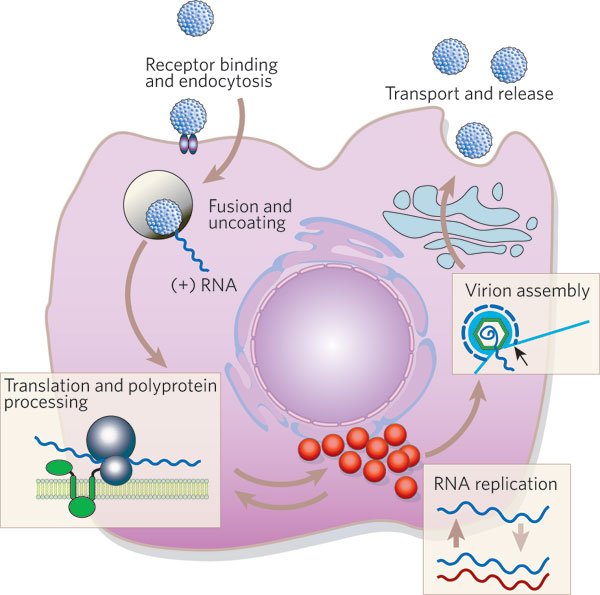
HCV genome consists of 9600 base pairs (bp). The 5′ and 3′ ends of the RNA is flanked by untranslated regions (UTRs), that are not translated into proteins but are important for translation and replication of the viral RNA. The 5' UTR has a [ribosome](http://en.wikipedia.org/wiki/Ribosome) binding site, i[nternal ribosome entry site](http://en.wikipedia.org/wiki/Internal_ribosome_entry_site) (IRES), which is folded into four stem-loop motifs which are called as I, II, III and IV. IRES is important for genome positioning on the host 40S ribosomal subunit to start the cap independent translation of viral RNA ***(Jubin, 2001).***

HCV encodes a single precursor polyprotein that is approximately 3010 amino acids in length This polyprotein is co and post-translationally processed by viral and cellular proteases into three structural proteins (core, envelope proteins E1, E2) and six non-structural viral proteins (, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) and a small membrane protein viroporin p7. Both envelope proteins (E1 and E2) are highly glycosylated and important in cell entry. E1 serves as the fusogenic subunit and E2 contains receptor-binding site for CD-81, a receptor expressed on hepatocytes and B-lymphocytes that acts as a receptor for HCV ***(Mizushima et al., 1994).***

Non-structural proteins function as helicase, protease, and RNA-dependent RNA polymerase. One region within NS5A is linked to interferon (IFN) response and is called the IFN sensitivity-determining region ***(Major and Feinstone, 1997)*.**

**(B) Life cycle**

The virus replicates mainly in the [hepatocytes](http://en.wikipedia.org/wiki/Hepatocyte) of the liver, where it is estimated that daily each infected cell produces approximately fifty virions (virus particles) with a calculated one trillion virions generated. The virus may also replicate in [peripheral blood mononuclear cells](http://en.wikipedia.org/wiki/Peripheral_blood_mononuclear_cell), potentially accounting for the high levels of [immunological disorders](http://en.wikipedia.org/wiki/Immunology) found in chronically infected HCV patients. HCV has a wide variety of [genotypes](http://en.wikipedia.org/wiki/Genotype) and mutates rapidly due to a high error rate of the virus' [RNA-dependent RNA polymerase](http://en.wikipedia.org/wiki/RNA-dependent_RNA_polymerase) and due to high replication rate. The mutation rate produces so many variants of the virus. These variants are considered [quasispecies](http://en.wikipedia.org/wiki/Quasispecies_model) rather than a conventional virus species **(fig.5 page25) *(Bartenschlager and Lohmann, 2000).***



***Fig (5): Life cycle of HCV (Lindenbach and  Rice, 2005).***

**HCV Life cycle:**

1) The virus locates and attaches itself to a liver cell. Entry into host cells occurs through complex interactions between virions proteins, present on its protective lipid coat and cell-surface molecules [CD81](http://en.wikipedia.org/wiki/CD81), [LDL receptor](http://en.wikipedia.org/wiki/LDL_receptor), [claudin-1](http://en.wikipedia.org/wiki/CLDN1) and [occludin](http://en.wikipedia.org/wiki/Occludin).

2) HCV merges its lipid coat with the cell outer membrane. Once the lipid

coat has successfully fused to the plasma membrane, the membrane engulfs the virus - and the viral core is inside the cell.

3) The protein coat dissolves to release the viral RNA in the cell. The viral RNA then bind the cell's ribosomes, and begins the production of materials necessary for viral reproduction. Because hepatitis C stores its information in a "sense" or positive strand of RNA,

**N.B** the viral RNA itself can be directly read by the host cell's ribosomes, functioning like the normal mRNA present in the cell. As it begins producing the materials coded in its RNA, the virus also possibly shuts down most of the normal functions of the cell, conserving its energy for the production of viral material, although it occasionally appears that hepatitis C will stimulate the cell to reproduce (presumably to create more cells that can produce viruses). This explains why hepatitis C is often associated with liver cancer.

5)The viral RNA creates an antisense or negative version (the paired opposite) of itself as a template for the creation of new viral RNA. The viral RNA is now copied hundreds or thousands of times, making the genetic material for new viruses. Some of this new RNA will contain mutations.

6) The completed core assembles around the new viral RNA into new viral particles. The completed particle is called a nucleocapsid.

7) The new viruses travel to the inside portion of the plasma membrane and attach to it, creating a bud. The plasma membrane encircles the virus and then releases it, providing the virus with its lipid coat, which it will later use to attach to another liver cell [***(Bartenschlager***](http://vir.sgmjournals.org/search?author1=Ralf++Bartenschlager&sortspec=date&submit=Submit) ***and*** [***Lohmann***](http://vir.sgmjournals.org/search?author1=Volker++Lohmann&sortspec=date&submit=Submit)***, 2000).***

Like all viruses, the hepatitis C virus is gradually inactivated outside the body of a host. The presence of heat can have a drastic impact on the virus's life span outside the body. The virus can remain infectious outside a host for about sixteen days at 25°C and two days at 37°C, while it can remain active for more than six weeks at temperatures less than or equal to 4°C. When heated to temperatures of 60°C and 65°C, the hepatitis C virus can be inactivated in eight and four minutes, respectively ***(Song et al., 2010).***

Based on genetic differences between HCV isolates, the hepatitis C virus species are classified into six [genotypes](http://en.wikipedia.org/wiki/Genotypes) (1-6) with several subtypes within each genotype (represented by letters). Subtypes are further broken down into quasispecies based on their genetic diversity. The predominance and distribution of HCV genotypes varies globally ***(Simmonds et al., 2005).***

Each HCV genotype is unique with respect to its nucleotide sequence, geographic distribution, and response to therapy ***(Jubin, 2001).***

HCV genotype 4 (HCV-4) is common in the Middle East and in Africa, where it is responsible for more than 80% of HCV infections ***(Dubuisson, 2007).*** HCV-4 is responsible for almost 90% of infections in Egypt and is considered a major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma, and liver transplantation in the country ***(Kohaar et al., 2010).***

HCV genotype 4 is a very heterogeneous genotype showing significant genetic divergence and more subtypes compared with other genotypes. To date, 18 subtypes (subtypes 4a, 4c, 4d, 4e, 4f, 4g, 4h, and 4k-4u) have been identified in different geographic regions ***(Rapicetta et al., 1998).***

HCV-4asubtype is predominant in Egypt representing 55% of HCV ***(Kamal and Nasser, 2008).*** Molecular evolutionary analysis on Egyptian genotype 4a isolates indicates that the Egyptian HCV epidemic was initiated and propagated by extensive antischistosomiasis mass treatment campaigns, during which tartar emetic (potassium antimony tartarate) was administered as a series of intravenous injections using improperly sterilized syringes and needles ***(Pybus et al., 2003)***.

Although the anti-schistosomal campaigns were terminated in the early 1980s, the prevalence and incidence of HCV remains high in Egypt. Thus, it seems that the status of HCV in Egypt is not only a consequence of the anti-schistosomal therapy but also due to new infections acquired beyond that era ***(Zakaria et al., 2007).***

**3**- **Epidemiology**

Hepatitis C is a disease with a significant global impact. According to the World Health Organization (WHO) there are 130-170 million people infected with HCV, corresponding to 2-2.5% of the world’s total population, there are considerable regional differences in some countries ***(WHO, 2011).***

Egypt has the highest prevalence of antibodies to hepatitis C virus (HCV) in the world, estimated nationally at 14.7%. About 9.8% of populations are chronically infected ***(Miller and Abu-Raddad, 2010).***

**4**- **Mode of transmission**

The primary route of transmission in the [developed world](http://en.wikipedia.org/wiki/Developed_world) is [intravenous drug use](http://en.wikipedia.org/wiki/Intravenous_drug_use) (IDU), while in the [developing world](http://en.wikipedia.org/wiki/Developing_world) the main methods are [blood transfusions](http://en.wikipedia.org/wiki/Blood_transfusions) and unsafe medical procedures ***(Maheshwari and Thuluvath, 2010).*** The cause of transmission remains unknown in 20% of cases ***(Pondé, 2011).***

**(A) Healthcare exposure**

[Blood transfusion](http://en.wikipedia.org/wiki/Blood_transfusion), transfusion of blood products and [organ transplantation](http://en.wikipedia.org/wiki/Organ_transplantation)  without HCV screening carry significant risks of infection. Those who have experienced a [needle stick injury](http://en.wikipedia.org/wiki/Needle_stick_injury) from someone who was HCV positive have about a 1.8% chance of subsequently contracting the disease themselves ***(Wilkins et al., 2010)*.**

  There is a risk from mucosal exposures to blood; but this risk is low, and there is no risk if blood exposure occurs on intact skin. Hospital equipment has also been documented as a method of transmission of hepatitis C including: reuse of needles and syringes, multiple-use of medication vials, infusion bags, and improperly sterilized surgical equipment ***(Alter, 2007).***

**(B) Sexual transmission**

Hepatitis C transmission through sexual activity is controversial. Sexual transmission of HCV is considered rare. Studies show the risk of sexual transmission in heterosexual, monogamous relationships is extremely rare or even nil. However, theCenters for Disease Control and prevention (CDC) do recommend the use of condoms between long-term monogamous partners ***(Vandelli et al., 2004)***. Sexual practices that involve higher levels of trauma to the anogenital mucosa do present a risk ***(Tohme and Holmberg, 2010)***.

**(C) Shared personal items**

Sharing Personal-care items such as razors, toothbrushes, and manicuring or pedicuring equipment can be contaminated with blood and may lead to HCV transmission.  HCV cannot spread through casual contact, such as hugging, kissing, or sharing eating or cooking utensils ***(Lock et al., 2006).***

**(D) Vertical transmission**

[Vertical transmission](http://en.wikipedia.org/wiki/Vertical_transmission) of hepatitis C from an infected mother to her child occurs in less than 10% of pregnancies ***(Lam et al., 2010).***  It is not clear when during pregnancy, transmission occurs, but it may occur both during gestation and at delivery ***(Pondé, 2011).***  A long labor is associated with a greater risk of transmission ***(Alter, 2007).*** There is no evidence that [breast-feeding](http://en.wikipedia.org/wiki/Breast-feeding) spreads HCV; however, to be cautious, an infected mother is advised to avoid breastfeeding if her nipples are cracked and bleeding ***(Mast, 2004).*** Perinatal transmission is observed in 3.2% of children born to mothers infected with HCV. The risk of perinatal transmission of HCV is higher estimated at 7.9% in children born to mothers co-infected with HIV and HCV ***(Bonacini and Puoti, 2000).***

**5- Pathogenesis**

Both humoral and cell-mediated immune responses participate in the host defense against HCV infection, but it is recognized that cell mediated response to the cytokine system plays a role in the immunopathogenesis of chronic hepatitis C ***(Jacobson Brown and Neuman, 2001).***

**6- Clinical picture**

**(A) Hepatic manifestations:**

**1) Acute hepatitis**:

The majority of newly infected patients will be asymptomatic and have a clinically non-apparent or mild course. Jaundice as a clinical feature of acute hepatitis C will be present in less than 25% of infected patients, therefore, acute hepatitis C will not be noticed in most patients. Periodic screening for infection may be warranted in certain groups of patients, who are at high risk for infection. Other symptoms that may occur are similar to those in other forms of acute viral hepatitis, including malaise, nausea, and right upper quadrant pain. Fulminant hepatic failure due to acute HCV infection is very rare; it may be more common in patients with underlying chronic hepatitis B virus infection ***(Chu et al., 1999)*.**

**2) Chronic hepatitis**:

 Most of patients experience minimal or no symptoms during the initial few decades of the infection but chronic hepatitis C may be associated with fatigue ***(Lauer and Walker, 2001).*** About 80% of those exposed to the virus develop a chronic infection ***(Nelson et al., 2011).***

About 10–30% of people with chronic infection develop cirrhosis over 30 years. Cirrhosis is more common in those co-infected with hepatitis B or HIV, [alcoholics](http://en.wikipedia.org/wiki/Alcoholic) and those of male gender. Those who develop cirrhosis have a 20-fold greater risk of [hepatocellular carcinoma](http://en.wikipedia.org/wiki/Hepatocellular_carcinoma), a rate of 1–3% per year ***(Wilkins et al., 2010).***

If this is complicated by excess alcohol the risk becomes 100 fold greater ***(Mueller et al., 2009).***  Liver cirrhosis may lead to [portal hypertension](http://en.wikipedia.org/wiki/Portal_hypertension), [ascites](http://en.wikipedia.org/wiki/Ascites) , [easy bruising or bleeding](http://en.wikipedia.org/wiki/Coagulopathy), varices, [jaundice](http://en.wikipedia.org/wiki/Jaundice), and a syndrome of cognitive impairment known as [hepatic encephalopathy](http://en.wikipedia.org/wiki/Hepatic_encephalopathy). It is a common cause for requiring a [liver transplant](http://en.wikipedia.org/wiki/Liver_transplant) ***(Ozaras and Tahan, 2009).*** Hepatitis C after many years becomes the primary cause of [cirrhosis](http://en.wikipedia.org/wiki/Cirrhosis) and [liver cancer](http://en.wikipedia.org/wiki/Liver_cancer) ***(Rosen, 2011).***

**(B) Extrahepatic manifestations:**

Hepatitis C is also rarely associated with [Sjögren's syndrome](http://en.wikipedia.org/wiki/Sj%C3%B6gren%27s_syndrome) (an autoimmune disorder), [thrombocytopenia](http://en.wikipedia.org/wiki/Thrombocytopenia), [lichen planus](http://en.wikipedia.org/wiki/Lichen_planus), [diabetes mellitus](http://en.wikipedia.org/wiki/Diabetes_mellitus), and B-cell [lymphoproliferative disorders ***(Zignego et al., 2007)***.](http://en.wikipedia.org/wiki/Lymphoproliferative_disorder) Thrombocytopenia occurs in some patients with chronic hepatitis C ***(Louie et al., 2011)***.

**7- Diagnosis**

**(A) Clinical diagnosis of HCV virus:**

Common symptoms of hepatitis C as fatigue, muscle ache, loss of appetite or nausea are unspecific. In many cases, these symptoms are mild or not present. Consequently, hepatitis C is often diagnosed accidentally. It is estimated that only 30-50% of individuals infected with HCV are aware of their disease ***(Deuffic-Burban et al., 2010****)****.***

Untreated hepatitis C advances to a chronic state in up to 80% of people, which leads to liver cirrhosis in 20-40% with an accompanying risk of hepatic decompensation, hepatocellular carcinoma and death. HCV diagnostics should be performed thoroughly in all patients presenting with increased aminotransferase levels, with chronic liver disease of unclear etiology and with a history of enhanced risk of HCV transmission ***(McHutchison, 2004).***

**(B) Laboratory diagnosis of HCV virus**

**1. Liver enzymes tests:**

There are two liver enzymes used in the evaluation of patients with HCV infection. These are alanine aminotransferase (ALT), and aspartate aminotransferase (AST). In general, it is clear that the presence of obesity and female gender can affect the level of ALT ***(Koff and Younossi, 2004).***

Liver enzyme levels can fluctuate over time, and the presence of one normal value is not sufficient to determine ALT level. Liver histology may not always correlate with ALT values. HCV-infected patients with normal ALT values appear to have liver disease that is at an earlier histological stage and less active, however, 25% to 30% of such patients have significant histological fibrosis, with 5% to 10% having bridging fibrosis or cirrhosis. The absence of any elevation does not rule out significant injury or hepatic fibrosis, Liver enzyme tests do not reveal the cause of hepatic injury or reflect the true status of hepatic function ***(Sarbah and Younossi, 2000).***

**2. Laboratory tests**

Laboratory studies can provide useful values to predict the progression of liver disease. Laboratory studies should include a complete blood count (CBC), prothrombin time (PT), international normalized ratio (INR). Although the serum aminotransferase level correlates poorly with liver histology ***(Bhatty et al., 2009)***, the ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT) >1 is a dependable marker for cirrhosis ***(Hadziyannis et al., 2004)***. Increased INR and thrombocytopenia is also seen more frequently in cirrhosis ***(Adinolfi et al., 2001).***

**3. HCV antibody tests**

There are two types of tests to evaluate HCV antibody:

**a- The enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA).**

EIA is the initial serologic test used for HCV screening, its sensitivity and specificity are excellent, and its positive predictive value in high-risk patients is quite high, a patient with a positive EIA is considered to have HCV infection until proven otherwise. EIAs cannot distinguish between resolved and active infection. HCV antibodies usually become detectable 8 weeks following exposure. False positive results are rare now, but they were common with earlier generations of these assays, when false positive results do occur, they usually do so in patients with autoimmune liver disease or hypergammaglobulinemia who have normal liver enzymes. False negatives are also uncommon, when they do occur; they do so in immunosuppressed patients (e.g., organ transplant recipients and HIV-positive patients) and in patients on long-term hemodialysis ***(Pawlotsky, 2002)*.**

ELISA-3 tests are now the most widely used screening tests for HCV ***(Busch et al., 1992)***. Despite the improved specificity, confirmation of positive results is still required ***(Lauer and walker, 2001****).*

**b- The recombinant immunoblot assay (RIBA).**

The first generations of EIA tests were plagued by false positive results, so the researchers developed the RIBA as a supplemental semi quantitative assay to refine the specificity of positive anti-HCV EIAs. RIBA can identify false-positive EIA results that are sometimes seen in patients with no apparent risk factors for HCV infection and in patients with other immune system-mediated diseases, such as rheumatoid arthritis, however, RIBAs are becoming obsolete because their function can be performed better by HCV RNA testing ***(Singer and Younossi, 2001).***

**4. Virus detection tests (molecular biology based tests):**

Real- time PCR techniques such as TaqMan have been developed. These assays are rapid, sensitive and have broad dynamic ranges, provide precise quantitation of viral load ***(Morris et al., 1996).***

The HCV RNA test measures the amount of HCV RNA in the blood via target amplification with reverse transcriptase (RT) polymerase chain reaction (PCR), transcription- mediated amplification (TMA), or a signal amplification technique such as a branched DNA (b-DNA) assay ***(Pawlotsky et al., 2002).*** HCV [RNA](http://en.wikipedia.org/wiki/RNA) can be detected by PCR typically one to two weeks after infection ***(Ozaras and Tahan, 2009).***

|  |  |  |
| --- | --- | --- |
| **HCV antibody tests results** | **Virus Detection Tests results** | **Interpretation** |
| Positive Anti-HCV | PositiveHCV RNA | Acute or chronic HCV depending on the clinical context. |
| Positive Anti-HCV | Negative HCV RNA | 1- Resolution of HCV.  2- Acute HCV during period of low-level viremia. |
| Negative Anti-HCV | Positive HCV RNA | 1- Early acute HCV infection.  2- Chronic HCV in immunosuppressed  3- False positive HCV RNA test. |
| Negative Anti-HCV, | Negative HCV RNA | Absence of HCV. |

***Table (2): Interpretation of HCV assays*** ***(Ghany et al., 2009).***

**5. HCV genotyping**

Genotyping can be performed by different methods which are based on amplification with the PCR assay, currently, the reverse hybridization line probe assay (LIPA, Bayer Diagnostics, and Emeryville, CA) is the most commonly used genotyping method in practice ***(Rossau et al., 1993).***

Genotyping can also be performed serologically. The techniques are based on the detection of antibodies directed to genotype-specific HCV epitopes generally located in the NS4 and /or core proteins, two assays have been developed based on competitive EIA or immunobloting ***(Dixit et al., 1995).***

Genotype is highly associated with response to viral therapyand is essential in determining the optimal treatment duration ***(Shiffman et al., 2007)****.*

**6. Radiographic studies**

Several modalities of radiographic tests are used to evaluate patients with chronic HCV. Ultrasonograohy is useful in the evaluation for evidence of portal hypertension such as splenomegaly, recanalization of the umbilical vein and ascites. It is also useful in the screening of hepatocellular carcinoma (HCC) although Computeriezed tomography (CT) scan or Magnetic resonance imaging (MRI) provides higher sensitivity for this purpose. Liver-Spleen scans can provide additional information when cirrhosis is suspected ***(Harisinghani and Hahn, 2002).***

**7. Biopsy**

There are three primary reasons for performing a liver biopsy: it provides helpful information on the status of the liver injury, it identifies features useful in the decision to embark on therapy and it may reveal advanced fibrosis or cirrhosis that necessitates surveillance for (HCC) and/or screening for varices. The biopsy is assessed for grade and stage of the liver injury, but also provides information on other histological features that might have a bearing on liver disease progression like hepatic steatosis; a common presentation in HCV infected patients ***(Kleiner, 2005)***

The procedure is not without risks including pain, bleeding and perforation of other organs ***(Dienstag, 2002).*** The liver biopsy has been widely regarded as the “gold standard” for defining the liver disease status, but it has drawbacks that have prompted questions about its value ***(Crockett et al., 2006).***

Efforts are underway to seek alternative means of establishing information on the extent of fibrosis by focusing on noninvasive blood marker panels. These markers are useful for establishing the two ends of the fibrosis spectrum (minimal fibrosis and cirrhosis) but are less helpful in assessing the mid-ranges of fibrosis or for tracking fibrosis progression ***(Rockey and Bissell, 2006)***.

The recently developed elastography that uses ultrasound and low frequency elastic waves to measure liver elasticity ***(Sandrin et al., 2003)*** has improved the ability to define the extent of fibrosis without a liver biopsy, particularly when combined with other noninvasive markers ***(Castera et al., 2005).*** It is not yet ready to replace the liver biopsy since the failure rate is higher in obese patients, and there is now evidence that the transient elastography score can be unexpectedly increased in persons with acute hepatitis who have high necroinflammatory activity but no or minimal fibrosis ***( Sagir et al., 2008).***

### METAVIR Score is commonly used for staging of fibrosis and activity. Fibrosis is graded on a 5-point scale from 0 to 4. The activity, which is the amount of necro-inflammation, is graded on a 4-point scale from A0 to A3 *(Bedossa, and Poynard, 1996).*

**8- Prevention**

A major obstacle to vaccine development is the extensive genetic variation between different strains and [genotypes](http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index7.html#g), and even the significant [antigenic](http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index7.html#a) variation among virus quasispecies within individual patients ***(Abrignani et al., 1999).*** As there is no available vaccine against hepatitis C, the only means of protection are the implementation universal precautionsand safe injection practices. Screening and treatment of blood products is the only way to prevent transfusion-associated cases ***(van der Poel, 1999).*** Blood banks should discard donor units with elevated liver [enzyme](http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index7.html#e) levels (ALT and/or AST) even after the test for anti-HCV has been established For the prevention of posttransfusion hepatitis, it is important***.***that blood transfusions should be given only when necessary ***(Shepard et al., 2005).***

**9-** **Treatment of chronic hepatitis C**

The currently recommended therapy of chronic HCV infection is the combination of a pegylated interferon alpha and ribavirin. The choice of this regimen was based upon the results of three clinical trials that demonstrated the superiority of this combination treatment over standard interferon alpha and ribavirin ***(Hadziyannis et al., 2004).***

**(A) Historical review**

During research to produce a more efficient [vaccine](http://en.wikipedia.org/wiki/Vaccine) for [smallpox](http://en.wikipedia.org/wiki/Smallpox), ***Yasu-ichi*** ***Nagano*** and ***Yasuhiko Kojima*** noticed inhibition of viral growth in an area of rabbit-skin or testis previously [inoculated](http://en.wikipedia.org/wiki/Inoculate) with UV-inactivated virus. They hypothesised that some "viral inhibitory factor" was present in the tissues infected with virus and attempted to isolate and characterize this factor from tissue [homogenates](http://en.wikipedia.org/wiki/Homogenization_%28biology%29) (***Nagano and Kojima, 1954).*** After Nagano and Kojima separated the viral inhibitory factor from the viral particles using ultracentrifugation, they confirmed its antiviral activity lasted 1–4 days and did not result from [antibody](http://en.wikipedia.org/wiki/Antibody) production; their findings were published in 1958 (***Nagano and Kojima, 1958).***

The purification of interferons did not occur until 1978. A series of publications from the laboratories of [***Sidney Pestka***](http://en.wikipedia.org/wiki/Sidney_Pestka) ***and Alan Waldman between 1978 and 1981***, described the purification of the type I interferons IFN-α and IFN-β (***Pestka, 2007)***. By the early 1980s, the genes for these interferons were cloned allowing for the first time definitive proof that interferons really were responsible for interfering with viral replication (***Weissenbach et al., 1980).*** Gene cloning also confirmed that IFN-α was encoded by a family of related genes (***Nagata et al., 1980)***. Using [recombinant DNA technology](http://en.wikipedia.org/wiki/Recombinant_DNA_technology), interferon [gene](http://en.wikipedia.org/wiki/Gene) was inserted into [bacteria](http://en.wikipedia.org/wiki/Bacterium) in 1980 allowing mass cultivation and purification from [bacterial cultures](http://en.wikipedia.org/wiki/Microbiological_culture#Bacterial_culture) (***Nagata et al., 1980).***

## (B) Types of interferon

Based on the type of receptor through which they signal, interferons have been classified into three major types.

**1)** [**Interferon type I**](http://en.wikipedia.org/wiki/Interferon_type_I): Bind to a specific cell surface receptor complex known as the [IFN-α](http://en.wikipedia.org/wiki/IFN-%CE%B1) receptor ([IFNAR](http://en.wikipedia.org/wiki/Interferon-alpha/beta_receptor)) that consists of [IFNAR1](http://en.wikipedia.org/wiki/IFNAR1) and [IFNAR2](http://en.wikipedia.org/wiki/IFNAR2) chains. The type I interferons present in humans are [IFN-α](http://en.wikipedia.org/wiki/IFN-%CE%B1), [IFN-β](http://en.wikipedia.org/wiki/IFN-%CE%B2) and [IFN-ω](http://en.wikipedia.org/wiki/Interferon_type_I#IFN-.CF.89) *(****Liu, 2005).***

**2)** [**Interferon type II**](http://en.wikipedia.org/wiki/Interferon_type_II): In humans, this is [IFN-γ](http://en.wikipedia.org/wiki/IFN-%CE%B3), binds to [IFN- γ](http://en.wikipedia.org/wiki/IFN-%CE%B1)  receptor ([IFNGR](http://en.wikipedia.org/wiki/IFNGR)).

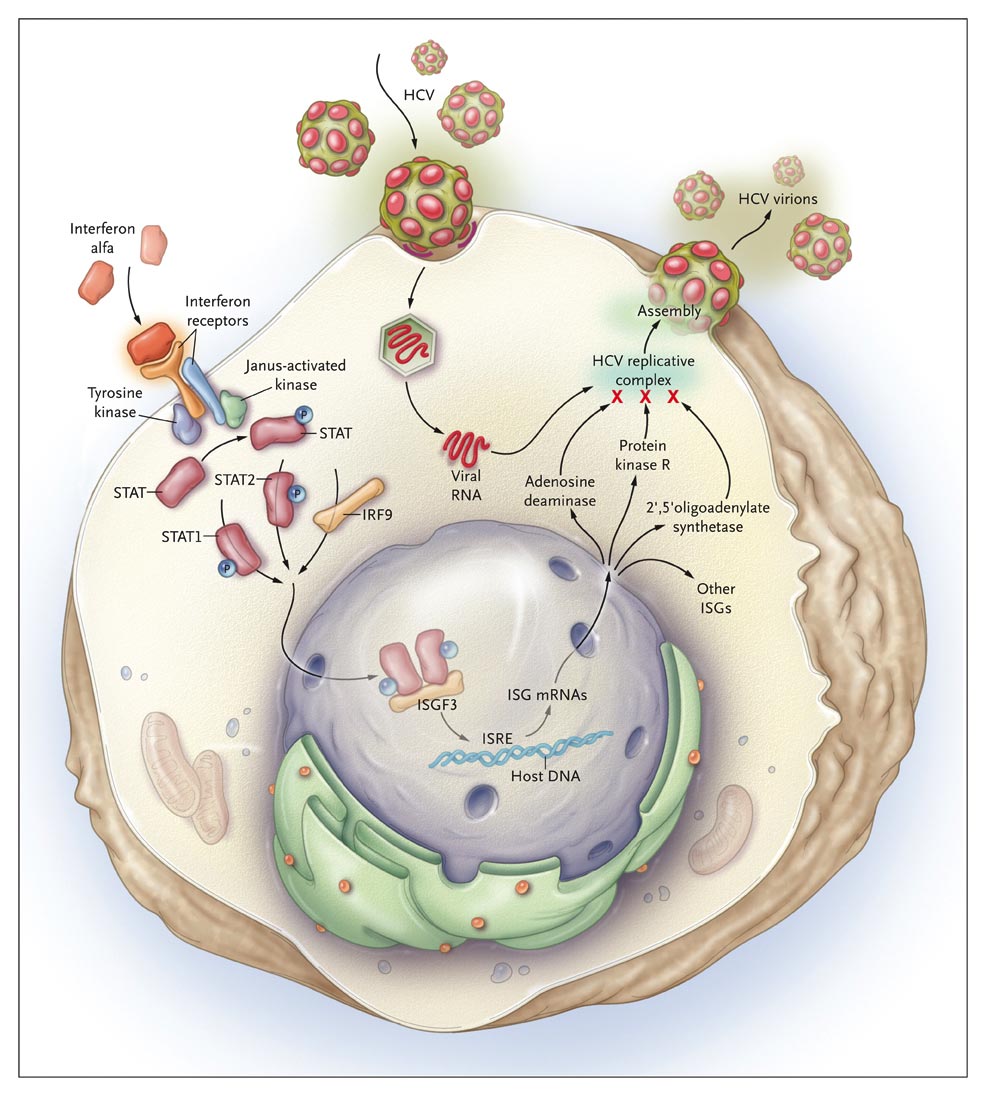
**3)** [**Interferon type III**](http://en.wikipedia.org/wiki/Interferon_type_III): Signal is through a receptor complex. It is not currently included in [Medical Subject](http://en.wikipedia.org/wiki/Medical_Subject_Headings)  ***(Vilcek, 2003).***

## (C) Mechanism of action of interferon

## Interferon alfa has potent antiviral and antiproliferative properties, but does not act directly against HCV. Instead, interferon alfa creates a non-specific antiviral environment within the hepatocyte by inducing interferon-stimulated genes. As an infected cell dies from a [cytolytic](http://en.wikipedia.org/wiki/Cytolysis) virus, viral particles are released that can infect nearby cells. However, the infected cell can warn neighboring cells of a viral presence by releasing interferon (IFN). Type I IFNs achieve their potent antiviral effects through the regulation of hundreds of IFN-stimulated genes (ISGs) *(Sen, 2001)*. IFNs induce ISGs transcription by activating the Janus kinase - signal transducer and activator of transcription pathway (Jak- STAT) (fig. 6) *(Darnell et al., 1994)*.

Type I IFNs bind to the same cell surface receptor (IFNAR) and activate the receptor-associated tyrosine kinases Jak1 and Tyk2. The kinases then phosphorylate and activate STAT1 and STAT2. The activated STATs translocate to the nucleus, where they bind specific DNA elements in promoters of ISGs. Many ISGs have antiviral activity but some are involved in other processes such as lipid metabolism, apoptosis, protein degradation, and inflammatory cell responses ***(de Veer et al., 2001).***

The neighboring cells, in response to interferon, produce large amounts of an [enzyme](http://en.wikipedia.org/wiki/Enzyme) known as [protein kinase R](http://en.wikipedia.org/wiki/Protein_kinase_R) (PKR). This enzyme [phosphorylates](http://en.wikipedia.org/wiki/Phosphorylation) a protein known as [eukaryotic](http://en.wikipedia.org/wiki/Eukaryotic) translation initiation factor-2a ([eIF-2](http://en.wikipedia.org/wiki/EIF-2)) in response to new viral infections; eIF-2 forms an inactive complex with another protein, called [eIF2B](http://en.wikipedia.org/wiki/EIF2B), to reduce protein synthesis within the cell. Another cellular enzyme, [RNAse L](http://en.wikipedia.org/wiki/RNAse_L) also induced following PKR activation destroys RNA within the cells to further reduce protein synthesis of both viral and host genes. Inhibited protein synthesis destroys both the virus and infected host cells *(****Fensterl and Sen, 2009).*** Interferons also limit viral spread by increasing [p53](http://en.wikipedia.org/wiki/P53) activity, which kills virus-infected cells by promoting [apoptosis](http://en.wikipedia.org/wiki/Apoptosis) *(****Moiseeva et al., 2006).***

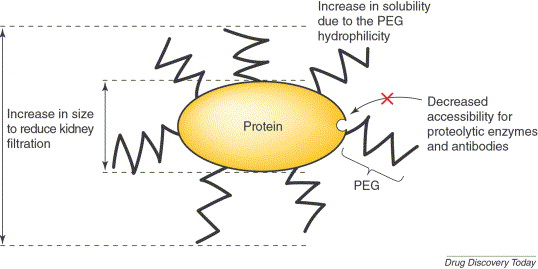


***Fig (6): Mechanism of action of interferon (***[***http://depts.washington.edu/hepstudy/hepC/mgmt/meds/discussion.html***](http://depts.washington.edu/hepstudy/hepC/mgmt/meds/discussion.html)***).***

**(D) Pegylation**

Pegylation is the process of covalent attachment of [polyethylene glycol](http://en.wikipedia.org/wiki/Polyethylene_glycol) (PEG) polymer chains to another molecule**(Fig. 7 page 43) *(Veronese and Harris, 2002)***.

In January 2001, the [Food and Drug Administration](http://en.wikipedia.org/wiki/Food_and_Drug_Administration) (FDA) approved the use of [pegylated](http://en.wikipedia.org/wiki/PEGylated) interferon-alpha in the United States of America (USA), initially used for production of [pegylated interferon-alpha-2b](http://en.wikipedia.org/wiki/Peginterferon_alfa-2b) (*Pegintron*). Approval for [pegylated interferon-alpha-2a](http://en.wikipedia.org/wiki/Peginterferon_alfa-2a) (*Pegasys*) followed in October 2002. These pegylated drugs are injected once weekly, rather than administering three times per week, as is necessary for conventional interferon-alpha ***(Jamall et al., 2008).***



***Figure (7): Pegylation (***[***Veronese***](http://www.sciencedirect.com/science/article/pii/S1359644605035750) ***and Pasut, 2005).***

***The benefits of covalent attachment of PEG to a drug or therapeutic protein are:***

* Mask the agent from the host's immune system (reduced [immunogenicity](http://en.wikipedia.org/wiki/Immunogenicity) and [antigenicity](http://en.wikipedia.org/wiki/Antigenicity)).
* Increase the hydrodynamic size (size in solution) of the agent, which prolongs its circulatory time by reducing [renal](http://en.wikipedia.org/wiki/Renal) clearance.
* Provide water solubility to [hydrophobic](http://en.wikipedia.org/wiki/Hydrophobic) drugs and proteins ***(Veronese and Harris, 2002)***.

The doses of these two forms of pegylated interferons differ. The optimal dose of peginterferon alfa-2b is 1.5 µg /kg/week plus a fixed dose of ribavirin (10.6 mg/kg per day). Peginterferon alfa-2a is administered at a fixed dose of 180 µg/week given subcutaneously together with ribavirin 1000 to 1200 mg daily (1000 mg for those who weight < 75 kg and 1200 mg for those who weight >75 kg) ***(Fried et al., 2002).*** Both pegylated interferons have comparable efficacy, although some smaller trials suggest slightly higher SVR rates in patients treated with pegylated interferon α-2a ***(Ascione et al, 2010)***.

The optimal duration of treatment should be based on the viral genotype. For patients with HCV genotype 4 infection, combination treatment with pegylated interferon plus weight based ribavirin administered for 48 weeks appears to be the most effective ***(Khuroo and Dahab, 2004).***

Evaluation should continue for at least 6 months after stopping therapy to assess whether the response to therapy is sustained ***(Fried and Hoofnagle, 1995).***

Combination therapy significantly reduces viral load, serum ALT activity, improves histological activity and blocks progression of fibrosis in patients who have not cleared HCV compared to the natural history of the disease. Patients who still have a positive HCV PCR after treatment should therefore no longer be called non responders to interferon. Interferon might even reduce the [incidence](http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index7.html#i) of HCC and mortality ***(Poynard et al., 1999).***

Ribavirin is a guanosine nucleoside analogue with broad antiviral activity in vitro against a range of RNA and DNA viruses. The exact mechanism of action against HCV remains incompletely understood, but presumably involves one or more of the following mechanisms:

* Enhanced host T-cell immune clearance of HCV.
* Inhibition of the host enzyme inosine monophosphate dehydrogenase (IMPDH), with depletion of pools of guanosine triphosphate (GTP), an essential substrate for viral RNA synthesis.
* Direct inhibition of HCV replication.
* RNA virus mutagenesis that drives HCV to error catastrophe ***(Feld and Hoofnagle, 2005).***

**(E) Factors affecting response to combination therapy**

Pretreatment predictors of response are useful for advising patients on their likelihood of response**(fig. 7)** ***(Manns et al., 2001).***

**Viral factors**

* **Genotype and quaspecies.**
* **Viral load.**
* **ace**
* **Body weight**
* **Patient compliance**

**Comorbidities**

**Host factors**

* **Age.**
* **Gender.**
* **Race.**
* **Body weight.**
* **Compliance.**
* **Comorbidities.**

**Treatment factors**

* **Dose and duration.**

***Fig. (8): Factors affecting response to combination therapy.***

**1) Host related factors**

* Age, gender, race and body weight.

Age less than 40 years, Female gender,body weight <75 kg and non–African-American race are associated with better response ***(Romero-Gomez et al., 2005).***

* Compliance.

The adherence of each patient to treatment is highly variable. Chances of achieving an SVR significantly decrease when patients receive <80% of the total dose of peg-IFN and/or <80% of total ribavirin and/or during <80% of the total period of treatment ***(McHutchison et al., 1998).***

* Comorbidities.

Comorbidities such as HIV and/or HBV co-infection, excess alcohol intake and drug use are generally associated with lower SVR rates ***(Alberti, 2009).*** Insulin resistance reduces the chances of achieving an SVR ***(Younossi and McCullough*, 2009*).*** Smokers suffering from CHC tend to have lower response to IFN-α compared to non-smokers ***(Pessione et al., 2001).***

The two common non-HCV conditions that might affect disease progression and possibly treatment response are excess hepatocellular iron ***(Olynyk et al., 1995)*** and steatosis ***(Rubbia-Brandt et al., 2004).*** Identifying either of these two features does not preclude initiating treatment, but their presence provides additional information regarding the likelihood of response to treatment ***(Westin et al., 2007).*** Recently, a polymorphism in the Interleukin- 28B (IL-28B) gene has been identified to be associated with virological response and may largely account for racial differences in treatment ***(Ge et al., 2009).***

**2) Viral related factors**

* Genotype and quaispecies.

Hepatitis C virus- genotype is the strongest baseline predictor of IFN response. Response rates for genotype 4 are (approximately 65%). In a recent study, the authors analyzed the amino acid sequence of about 100 patients before treatment with IFN plus ribavirin. They show that the HCV sequence of non-responders presents three folds more hydrophobic pairs of amino acids than the sequence of responders. These hydrophobic amino acids were predicted to contribute to IFN treatment failure by stabilizing the HCV proteins complex ***(Aurora et al., 2009)*.**

* Viral load.

Patients with a high viral load >800, 000IU/ml are less sensitive to the treatment than patients with viral load <800, 000 IU/ml ***(Jensen et al.,* *2006).***

**3) Treatment related factors**

The dose of (1.5 mg/kg/week) is associated with more favorable response than (0.5 mg/kg/week) ***(Romero-Gomez et al., 2005).***

**(F) Treatment of non responders**

Approximately thirty percent of patients treated with pegylated interferon and ribavirin are unable to clear virus from the serum ***(Manns et al., 2001).*** Options for non-responders to pegylated interferon and ribavirin are limited. Retreatment with the same regimen leads to an SVR in fewer than 5% of patients and therefore cannot be recommended ***(Cheruvattath et al., 2007).*** There is no evidence that switching to alternative interferons is effective ***(Cornberg et al., 2006).***

Maintenance therapy with peginterferon with the goal of delaying or preventing progression to cirrhosis and/or hepatic decompensation is currently being assessed in different trials ***(Lee et al., 2004).***

Non-responders to peginterferon and ribavirin with advanced fibrosis should follow American Association for the Study of Liver Diseases (AASLD) guidelines for screening for hepatocellular carcinoma (HCC) and varices. Patients with mild fibrosis (Metavir 1 or and Ishak 2) should be monitored without treatment ***(Taliani et al., 2006).***

**(G) Treatment of relapser**

In the majority of cases, virological relapse occurs within the first 12 weeks. Late relapse (beyond 24 weeks) is extremely uncommon. Patients with virological relapse are likely to respond to the same regimen given a second time but will still experience an unacceptable rate of relapse. 32-50% patients achieve a sustained virological response. Patients with higher fibrosis scores are less likely to achieve an SVR. Patients who do not achieve HCV RNA negativity at week 12 have only a 5% chance of achieving SVR ***(Jacobson et al., 2005).***

**(H) Side effects of combined therapy**

**1) Early Side effects**

They include (e.g.):

* Fever.
* Malaise.
* Tachycardia.
* Chills.
* Headache.
* Arthralgias.
* Myalgias.
* Fatigue.
* Apathy.
* Nausea , loss of appetite and loss of weight.
* [Erythema](http://en.wikipedia.org/wiki/Erythema), pain and hardness on the spot of injection ***(Bhatti and Berenson, 2007).***

**2) Neuropsychiatric side effects**

They include (e.g.):

* Irritability.
* Confusion.
* Apathy.
* Behavioral, mood and cognitive changes.
* Depression.
* Suicidal ideation.

Mechanisms of Neuropsychiatric side effects are poorly understood. Interferon is not thought to readily cross the blood-brain barrier ***(Shakil et al., 1996).***

**3) Immunomodulatory side effects**

They include (e.g.):

* Autoimmune thyroiditis leading to hypothyroidism or hyperthyroidism ***(Carella et al., 1995).***
* Type 1 diabetes mellitus.
* Hemolytic anemia.
* Thrombocytopenic purpura.
* Addison’s disease.
* Myasthenia gravis.
* Lupus-like syndrome.
* Autoimmune hepatitis ***(Fried, 2002).***

**4) Bone marrow** **depression**

The effect of IFN on bone marrow results in decreased granulocytes and thrombocytes ***(McHutchinson et al., 2007)***.

**(I) Absolute contraindications to combination therapy**

1) Hepatic decompensation ***(Forns et al., 2003).***

2) Autoimmune disease ***(Krause et al., 2003).***

3) Cardiac arrhythmias, ischemic heart disease and uncontrolled hypertension ***(Angulo et al., 1999)****.*

4) Anaemia ***(Fried, 2002)***.

5) Renal failure ***(Bruchfeld et al., 2003).***

6) Pregnancy***(Roberts and Yeung, 2002).***

7) Uncontrolled diabetes ***(Fried, 2002)***.

**(J) New agents for treating hepatitis C**

Currently, the most promising drugs against HCV infection (genotype 1) are protease inhibitors. They are inhibitors of the HCV non-structural (NS) 3/4A serine protease. NS3 protease plays an important role in the HCV life cycle by causing cleavage of HCV polyprotein at the NS3-NS4A ***(***[***Romano et al., 2012***](http://www.frontiersin.org/Journal/10.3389/fphar.2013.00114/full#B5)***).***

Telaprevir and Boceprevir were approved by the Food and Drug Administration (FDA) in May 2011 for the treatment of HCV genotype 1 in combination with peginterferon and ribavirin (triple therapy) ***(***[***Popescu et al., 2012***](http://www.frontiersin.org/Journal/10.3389/fphar.2013.00114/full#B4)***).*** However, despite improved response rates, protease inhibitors have incremental toxic effects, high costs, and many drug interactions ***(Pearlman, 2012)***.

Clinical trials using anti-miR-122 to treat patients infected with HCV (miravirsen, Santaris, Pharma A/S) have shown promising results ***(***[***Lindow***](http://jcb.rupress.org/search?author1=Morten+Lindow&sortspec=date&submit=Submit)***and*** [***Kauppinen***](http://jcb.rupress.org/search?author1=Sakari+Kauppinen&sortspec=date&submit=Submit) ***, 2012).***

Chapter (III)

Relationship between miRNA and chronic

hepatitis C

microRNAs are receiving growing attention because of numerous reports on their dysregulation in human diseases and their potential as diagnostic and therapeutic targets. Because of their stability and presence in almost all body fluids, miRNAs constitute a novel class of non-invasive biomarkers. Numerous studies have shown that aberrant miRNA expression is associated with the development and progression of various types of human cancer and therefore studies on circulating miRNA profiles largely focused on cancer ***(Brase et al., 2010).***

Several studies have demonstrated a relationship between HCV and miRNAs. An initial study demonstrated that silencing of Dicer, which thereby inhibits miRNA processing, inhibited HCV replication by seven folds ***(Randall et al., 2007).*** miRNAs may be used by host cells to control viral infection ***(Grassmann and Jeang, 2008).***

Viral RNAs and the miRNA machinery may interact in various ways. First, mammalian viruses encode miRNAs that can act on the control of both viral and cellular genes by repressing their expression. Second, cellular miRNAs may recognize viral RNAs and silence them, or control the expression of a cellular protein necessary for the virus life cycle. It has also been suggested that miRNAs may be an effector in the classical vertebrate innate immune system ***(Obbard et al., 2009).***

In contrast to cellular mRNAs, HCV translation is cap-independent and mediated by an internal ribosome entry site (IRES) located within the 5′ UTR. The structural proteins include the core protein, which forms the viral capsid, while the E1 and E2 glycoproteins mediate viral entry and fusion. The non-structural proteins are required for RNA replication and are cleaved from the HCV polyprotein by NS2 and NS3 viral proteases. Although mammalian miRNAs may not share incellular antiviral response, in several instances, they can have a profound suppressive effect on the replication of several pathogenic viruses. Considering that, the IFN system is a key component of the innate immune defense against viral infections ***(Wang and Fish, 2012).***

The possibility for IFNs to mediate its effect, at least partially through induction of miRNAs is interesting. Indeed, a microarray analysis of RNA derived from IFN α/ β or IFN γ stimulated cells identified ~30 miRNAs that were differentially modulated in response to the treatment. Functional studies on the relevant miRNAs revealed that over-expression of miR-296 led to a substantial inhibition of HCV replication in a human hepatoma cell line, indicating that the miRNAs had an antiviral effect against the virus. Also, interferon (IFN) beta has been reported to modulate the expression of several cellular miRNAs that are capable of inhibiting hepatitis C virus (HCV) replication and infection, because they have sequence-predicted targets within the HCV genomic RNA ***(Pedersen et al., 2007).***

**microRNA signatures in liver diseases**

**1- miRNAs are abundant and finely regulated in the liver**

One of the first clues of the existence of miRNAs in mammals came from studies on genetic alterations in liver tumors. An unusual transcript, named (hcr), was described and characterized as liver-specific, essentially non-coding, specifically nuclear, and processed by endonucleases in one of woodchuck liver tumors investigated in 1989 ***(Moroy et al., 1989).*** Later on, the hcr transcript was found to encompass the so-called ‘‘pri-miRNA” for miR-122***.*** miRNA-122 was later described as a liver specific miRNA and has been reported in mouse, woodchuck and human livers, in human primary hepatocytes, and in cultured liver derived cells ***(Chang et al., 2004).***

Thus, the liver displays a differential miRNA expression profile during its development. The liver contains many cell types including parenchymal cells (i.e. hepatocytes) and “non-parenchymal cells” which include endothelial cells, stellate cells, lymphoid cells, and biliary epithelial cells (cholangiocytes). Each cell type may have completely distinct miRNA expression profiles ***(Meng et al., 2007).***

**2- miRNAs and viral hepatitis**

***In (2007), Pedersen and colleagues*** demonstrated IFN-mediated modulation of the expression of numerous cellular miRNAs in the treatment of hepatocytes infected with HCV. Expression of 30 cellular miRNAs in hepatocytes was influenced by IFN-α, β or IFN-γ. Specifically miRNAs, miR-128 and miR-296, (having nearly perfect complementarity in their seed sequences with HCV RNA genomes), were upregulated. Importantly, these miRNAs are capable of inhibiting HCV replication and infection. This has opened the door to our understanding of novel host–defense mechanisms that exist in mammalian cells as well as the antiviral mechanisms employed by interferon.

**3- miRNAs as therapeutic targets for liver diseases**

Chemically engineered oligonucleotides, termed ‘antagomirs’, have been developed and proven to be efficient and specific silencers of endogenous miRNAs in mice ***(Krutzfeldt et al., 2005).*** The silencing effect was considerably sustained over time probably because of a long half-life of endogenous miRNAs ***(Kim, 2005).***

Over the past several years, strategies based on both synthetic and expressed activators of the RNAi pathway have proved efficient to inhibit viral replication bothin vitroand in vivo **(fig. 3 page 11)** ***(Ely et al., 2008).***

Study of miRNAs flourished during the decade after their discovery. It is now clear that miRNAs can potentially regulate every aspect of cellular activity, from differentiation and proliferation to apoptosis, and modulate a large range of physiological processes from developmental timing to organogenesis ***(Taganov et al., 2007).***

Further, creating artificial miRNAs with salutary effects by promoting the expression of beneficial gene products (e.g. tumor-suppressor proteins) or targeting viral genomes (e.g., molecules designed to specifically target HCV-genome sequences) may become part of our patient management and complement chemotherapy and antiviral treatments ***(Chen, 2009)***