

RNA INTERFERENCE AND ITS ROLE IN THE INHIBITION AND CURE OF HIV/AIDS

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Abstract: The HIV/AIDS epidemic kills about 2.1 million people around the world every year. Unfortunately, until now attempts to control and diminish further spread of the disorder have resulted in very limited success. Although highly active antiretroviral therapy (HAART) is now available as a treatment option, the rate of its success is limited because of drug resistance. In this context, a more efficient treatment is very much necessary to fight with this fatal disease. The RNA interference (RNAi) can be employed as one of the powerful methods for treatment of the disease as it can effectively silence gene expression in a sequence specific manner. The RNAi-mediated treatment is therefore a promising substitution for therapy of the global epidemic HIV/AIDS in the future. The main objective of this review is to focus on an in-depth analysis of RNAi and the principles underlying RNAi treatment, and it's also describes the molecular mechanisms of HIV infection, current treatment facilities available to patients as well as therapeutic applications of RNAi along with their limitations as to why the options are inadequate to give a safe and sound cure of HIV/AIDS.

Key words: HIV/AIDS, RNA interference, treatments, limitations

miRNA: HIV/AIDS gniyrix AvKvxi mviwētk| cāZeQi cāq 2.1 ŋgij qb Awaemxi gZi NiUtq _vK| ŋK'S GB e'wai we'wZ ŋbqSŷ Ges Kgvfbvi cāPōv tmB Zj bvq ZZUv mdj bq| hwi I eZŷvB Highly Active Antiretroviral Therapy (HAART) GKgwI cāBmva' ŋPikRmv mjeav, ŋK'S Drug Resistance Gi KviŷY GtZ mdj Zvi nvi mwgZ| GB tēŋŷZ, gvivZK GB e'wai mŷ_ GiUu I vi Rb' GKiu weKī ŋPikRmv e'e'v AZ'S-cāqRb| ZvB RNA interference (RNAi) GB tivMī ŋPikRmvi tŷŷŷ GKiu kw'kvj x c×iZ ŋtmtē e'envi nZ cvti thtnZiGuV Rxtbi Aŋf'e'w'ŷK Sequence Specific cāUvq bxiē Kti _vK| AZGe, wēkē'vcr we'Z HIV/AIDS-Gi tŷŷŷ RNAi Gi gva'tg ŋPikRmv GKiu Avkvcō weKī ŋPikRmv nZ cvti | GB chŷjvPbri gj j ŷŷ nj, RNAi c×iZi GKiu ŋbMŷ wētkŷ I RNAi ŋPikRmvi tēQtb gj Zēŷ| HIV mŷŷgtYi AvYweK c×iZ, tivMŷ' i Rb' cāBmva' eZŷv ŋPikRmv mjeav, RNAi Gi ŋPikRmv we'wMZ cāqM, Ges Zv' i mŷgē×Zv A_ŷr tKb GB ŋPikRmv c×iZ HIV/AIDS Gi ŋbivc' I mŷz AvŷivM'i tŷŷŷ hē_ō bq, G_ŷj I GLvfb wēk' fŷte Avŷj vPv Ki v nŷqŷQ|

Introduction

RNAi refers to the introduction of a double stranded RNA (dsRNA) homologous specifically to the transcript of a target gene. It results in null or hypomorphic phenotypes which help to control the expression of the target genes within the living cells. Two types of small RNA molecules are found *i.e.* microRNA (miRNA) and small interfering RNA (siRNA) which are central to the RNAi. As it is known that RNAs are the direct products of genes, and the small RNAs can bind to specific other RNAs which can turn on/off a gene's expression either by increasing or decreasing their activity (Hannon 2002), which is believed to be the central foundation of treating diseases with RNAi. RNAi has an important role in defending cells against parasitic genes *i.e.* viruses and transposons. The antisense RNA experiments lead to the discovery of Interfering RNA. In 1998, the American

scientists Andrew Fire (MIT) and Craig Mello (Harvard) published their discovery of this mechanism that can degrade specific messenger RNA (mRNA) (Fire *et al.* 1998). Subsequently, they were awarded the 2006 Nobel Prize in Physiology or Medicine. Now, RNAi is thought to be the most effective process and one step ahead to the next genetic revolution.

The process of silencing a gene using RNAi is initiated by the dsRNA helices which commence endogenously (originating in the cell) as siRNA into the cell and/or constructed exogenously (coming from infection by a virus with an RNA genome or laboratory constructions) by non-coding RNAs like miRNA (Waterhouse *et al.* 2001; Bartel 2004; Rana 2007). Basically, RNAi is the route of impeding a gene's expression by using dsRNA to cleave the gene's RNA transcript or in another direction preventing the RNA from being translated to a

protein (Engels and Hutvagner 2006). In recent times, the use of these interfering RNAs in therapeutic purpose has been proven to be fruitful in treating several drastic diseases by elevating the immune response of the host body (Sioud 2007; Lindsay 2008; Sonkoly *et al.* 2008). miRNA and siRNA can act in a similar manner either by blocking protein synthesis or accelerating cellular degradation.

The central theme of RNAi lies beneath a gene silencing pathway, commonly known as RNAi pathway, where long dsRNA (*ca.* 200 nucleotides long) plays a key role in the silencing of the target gene in a sequence-specific manner at the mRNA level. This RNA-dependent gene silencing process is controlled by the RNA-induced silencing complex (RISC). In the cell's cytoplasm short dsRNA molecules interact with the catalytic RISC component argonaute (Daneholt 2006). When the dsRNA is exogenous, the RNA is imported directly into the cytoplasm. The initiating dsRNA can also be endogenous, as in pre-miRNAs derived from RNA-coding genes in the genome. The primary transcripts from such genes are first processed to form the characteristic stem-loop structure of pre-miRNA in the nucleus and then exported to the cytoplasm. Thus, the two dsRNA pathways, exogenous and endogenous, converge at the RISC complex after Dicer activity (Bagasra and Prilliman 2004).

When this dsRNA enters into the RNAi pathway (Fire *et al.* 1998; Clemens *et al.* 2000; Worby *et al.* 2001), it is cleaved by an RNase III like enzyme known as *Dicer* into siRNA (*ca.* 20-25 nucleotide long) duplexes (Fig. 1). Unwinding of these siRNAs occurs inside the assembled endoribonuclease containing complexes *i.e.* RISCs by the action of ATP, although the uptake of siRNAs by RISC is not dependent on ATP. These are then guided by siRNAs towards the complementary mRNA molecules where they cleave and raze the cognate mRNA. The cleavage and destruction of cognate mRNA molecule take place near the middle of the region bound by the siRNA strand (Carol and Bryan 2005; Gregory, *et al.* 2005). Apart from RISC mediated RNAi, there is another pathway called RITS (RNA-Induced Transcriptional Silencing), where siRNAs are incorporated into a RITS complex instead of RISC (Fig. 1). RITS comprises a chromodomain protein (Chp1), Argonaute 1 (Ago1), a protein of unknown function, Tas3, as well as siRNA molecules. RITS targets specific chromosomal areas for silencing through the production of a repressive chromatin structure known as heterochromatin through histone methylation (Verdel *et al.* 2004; Irvine *et al.* 2006). RISC cleaves RNA in

trans, whereas RITS binds to chromatin and is thought to destroy newly produced RNAs in *cis*.

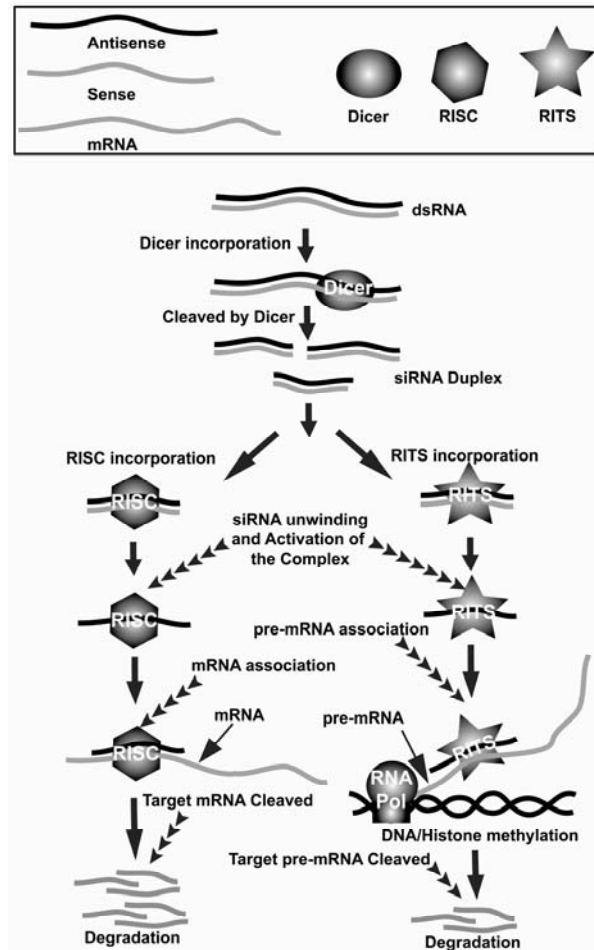


Fig. 1. The mechanisms of RNAi. RNAi pathway is triggered by the integration of dsRNA containing a sense (grey) and an antisense (black) strand. Subsequently, incorporation of Dicer (round) and cleavage of dsRNA in siRNA duplex occurs. Eventually, the siRNA duplex enters either into RISC or into RITS pathway to degrade the target mRNA (long grey).

In the field of modern molecular genetics research, the RNAi is now extensively used to silence or knock down the target gene. It is used not only in determination of gene function but also in therapeutic purposes including antiviral treatment of diseases (Fire *et al.* 1998; Hannon and Rossi 2004). Using RNAi pathway, some promising and potential studies have been conducted against several dreadful viruses like hepatitis B virus (HBV), hepatitis C virus (HCV), poliovirus, respiratory syncytial virus (RSV), influenza virus, and human papilloma virus

(Leonard and Schaffer 2005). The principal process is to prevent the establishment of prolific infection in susceptible cells by which these viruses enter into the blood stream (Carol and Bryan 2005). However, human immunodeficiency virus (HIV) was the first infectious agent targeted by RNAi, perhaps because the life cycle and pattern of gene expression of HIV is well-studied. Synthetic siRNAs and expressed shRNAs (short hairpin RNA) have been used to target virtually all of the HIV-encoded RNAs in cell lines, including *tat*, *rev*, *gag*, *pol*, *nef*, *vif*, *env*, *vpr*, and the long terminal repeat, LTR (Lee *et al.* 2002; Martinez *et al.* 2002, Novina *et al.* 2002; Jacque *et al.* 2002; Coburn and Cullen 2002). Although the use of RNAi in human cell lines is a quite new invention, it is gaining a lot of payback and thoughtful innovations on possible mechanisms in treating infection in the human cells by HIV and other viruses.

RNAi by which neurodegenerative disease genes such as those identified for spino-cerebellar ataxia type-1 (*SCA1* gene) and Huntington's disease (huntingtin gene) can be specifically silenced. These have great potential in becoming a successful therapeutic strategy for these diseases. Although it is still in the pre-clinical stages but clinical trials are currently on their way. In some other cases, the use of RNAi for therapeutic purposes in neurodegenerative diseases such as slow channel congenital myasthenic syndrome, fronto-temporal dementia, primary dystonia, amyotrophic lateral sclerosis (ALS), is producing some very promising results in pre-clinical trials (Sah 2006; Jagannath and Wood 2007). However, there exist some problems, such as toxicity due to non-specific silencing, generation of immune responses and over-saturation of RNAi pathway components that must be overcome in order to establish RNAi as a safe and effective therapy. Selective silencing of mammalian gene expression has recently been achieved using siRNAs also. There has been evidence of silencing viral gene expression in HPV (human papilloma virus) -positive human cervical carcinoma cells treated with siRNA, where the target was HPV E6 and E7 gene expression machinery by which the abnormal cell proliferation is driven by co-operative effects of these viral genes. The results indicate selective degradation of E6 and E7 mRNAs and the HPV-negative cells appeared to be unaffected by the anti-viral siRNAs (Jiang and Milner 2002). According to Kusov *et al.* 2006, the effect of siRNAs on severe course of HAV (hepatitis A virus) viral infection holds promise as a therapeutic tool as they have identified an exceptionally efficient siRNA target 2C-1 [GGUUGAAGUUAACC UGCUUU, nt 4796 to 4813; accession number M59808 augmented with known 5- and 3-terminal bases]. In the

HAV gene 2C this target significantly reduced the viral infectious titer without giving way to measurable viral escape mutants (Kusov *et al.* 2006). However, a retrovirus-based RNAi system was developed to drive expression and delivery of Hepatitis B virus (HBV)-specific short hairpin RNA (shRNA) in HepG2 cells. The levels of HBsAg and HBeAg and that of HBV mRNA were dramatically decreased by this RNAi system (Jia *et al.* 2006).

Probable origin and causes of HIV/AIDS

HIV is a lentivirus (a member of the retrovirus family) that causes AIDS (Weiss 1993; Douek *et al.* 2009), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. HIV might have originated from an analogous kind of virus called Simian Immunodeficiency Virus (SIV), which is found in a sub-group of chimpanzees *Pan troglodytes troglodytes* (Gao *et al.* 1999; Bailes *et al.* 2003). The virus emerged into the human population due to viral transfer between corresponding animals and the human, a process known as zoonosis. However, untreated HIV disease is characterized by a gradual deterioration of immune function where crucial immune cells $CD4^+$ T cells are disabled and killed during the typical course of infection. These cells, sometimes called T_H (helper) cells, play a vital role in the immune response, signaling other cells in the immune system to perform their special functions. Several factors contribute to the rapid transmission and infection of HIV/AIDS of which unprotected heterosexual contacts are most prominent. Some other minor contributors, which sometimes proved to be the prevalent cause of HIV transmission, include male homosexual contact, use of contaminated needles, sharing razors and even mouth-to-mouth deep kissing (Buvé 2000; Denning 2000; Katz *et al.* 2000). According to a UNAIDS, WHO, press release entitled *2007 AIDS epidemic update*, an estimated 33.2 million people lived with the disease worldwide, and it killed an estimated 2.1 million people including 330,000 children under 15 years and also each year approximately 3 million persons become newly infected with this deadly virus.

Mechanism of virus entry and transmission

Most particularly cell-free viral transmission occurs where viral propagation takes place *via* the binding of cell-free virions to a permissive host cell through CD4 and viral co-receptors (*CCR5* or *CXCR4*) (Fig. 2A). This is followed by viral entry into the cytoplasm by fusion and subsequent viral replication. In the early stage of

HIV disease, most people harbor viruses that use, in addition to $CD4^+$, the co-receptor $CCR5$ to enter their target cells. With disease progression, the spectrum of co-receptor usage expands in approximately 50 percent of patients to include other receptors, notably the molecule $CXCR4$. Virus that uses $CCR5$ is called R5 HIV and virus that uses $CXCR4$ is called X4 HIV.

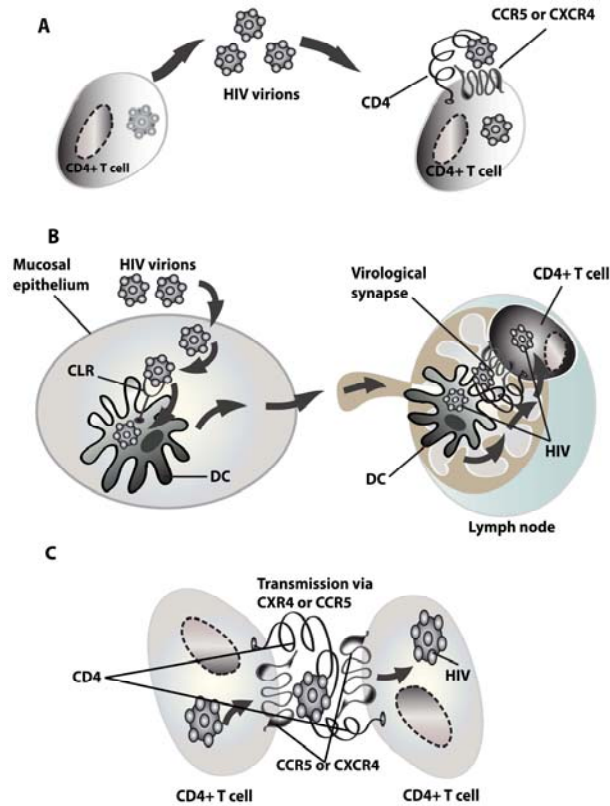


Fig. 2. Three mechanisms of HIV propagation (adapted from Piguet and Sattentau 2004). The arrows indicate the path of transmission. (A) Cell-free viral transmission. (B) DC-T cell viral transmission. (C) T cell-T cell viral transmission.

Another infection process occurs in the mucosal epithelia where DCs (dendritic cells) capture HIV virions *via* viral binding to C-type lectin-related (CLR) molecules or other cell surface receptors, without necessarily becoming infected, and represent infectious virus to $CD4^+$ T cells after migration to the lymph nodes (Fig. 2B). However, T cell-T cell viral transmission also occurs when an HIV-1-infected $CD4^+$ T cell infects a second $CD4^+$ T cell without the requirement for release of cell-free virions into the surrounding extracellular milieu, which represents viral propagation through direct cell-to-cell transmission (Fig. 2C).

The molecular mechanisms of HIV infection

The general structure of HIV is shown in Fig. 3 and the general infection process of HIV begins when the virus infects the host cell by binding to the membrane receptor $CD4^+$ (Fig. 4). After that, it penetrates immediately inside the host cell, makes a copy of its genetic material and incorporates it into the host cell DNA. Upon integration in to the genome of the host cell, the viral DNA replicates together with the host cell DNA. In the last stage of infection, the newly assembled virus particles disrupt and destroy the host cell by a process known as lysis and are released into the host's blood stream (Fig. 4) (Bukrinsky and Haffar 1997; Bukrinsky 2001).

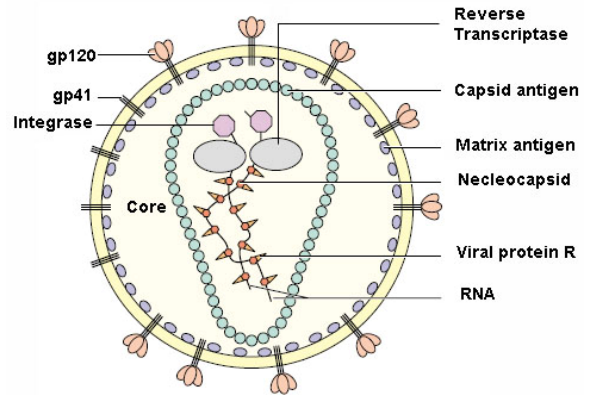


Fig. 3. A generalized structure of HIV (adapted from Bukrinsky 2001).

The molecular mechanisms of HIV infection have been described in detail by various workers such as Chan and Kim (1998) and Wyatt and Sodroski (1998). The entry to the cell begins through the interaction of the trimeric envelope complex ($gp160$ spike) and both $CD4$ and a chemokine receptor (generally either $CCR5$ or $CXCR4$) on the cell surface where $gp160$ spike contains binding domains for both $CD4$ and chemokine receptors. The primary binding of HIV happens between $CD4$ surface receptor of target cell and glycoprotein $gp120$ of the HIV surface. This encourages the secondary binding of HIV to the chemokine receptor, either $CCR5$ or $CXCR4$. This happens because during primary binding $gp120$ changes its conformation due to fusion with $CD4$ receptor, which allows the $gp41$ to be exposed and assisted in the secondary binding.

Following the secondary binding, a two-pronged attachment of HIV genome triggers the fusion of HIV membrane to the host cells. Once fused with the target cells, the viral genome (HIV RNA) and viral proteins (reverse transcriptase, integrase, ribonuclease and protease) are injected into the host cells (Chan and Kim 1998). The reverse transcriptase then liberates the

ssRNA (single stranded RNA) from the viral genome and makes a complimentary copy to produce viral dsDNA by the process of reverse transcription. The viral dsDNA is then incorporated into the host cell nucleus by the action of integrase. This integrated phase of HIV is called provirus, which may lie dormant in the latent phase of HIV infection (Zheng *et al.* 2005).

In the host cellular machinery, the transcriptional factors responsible for provirus activation is further stimulated by the environmental or internal stimuli. Among these, the transcriptional factor $NF-\kappa\beta$ is the most potential and prominent one which is available in the host machinery and is up regulated due to the T cell activation (Hiscott 2001). The incorporated provirus is copied to the mRNA, which in turn, is then spliced and produces the regulatory proteins *Tat* and *Rev*. When *Rev* accumulates steadily, the splicing of mRNA is inhibited and the unspliced mRNAs begin to assemble structural proteins *Gag* and *Env* in full swing. This allows the virus genome to produce new viral particles (Pollard and Malim 1998). The consequence of this whole process is the production of a large number of viral proteins and viral RNAs which are then assembled themselves to produce immature viruses. The latter are then bud out of the host and complete their maturation to infect and destroy other host cells (Fig. 4).

Current treatment facilities for HIV/AIDS

The current treatment option of the HAATR is supposed to hold a remarkable position in controlling the progression of HIV/AIDS (Palella *et al.* 1998). The Federal Drug Administration (FDA) has approved some generic formulations of antiretroviral drugs (e.g. didanosine, stavudine, zidovudine, azidothymidine) for the treatment of HIV infection. But the frontier of these Nucleoside Reverse Transcriptase Inhibitors (NRTIs) is drawn by the development of drug resistance in the host body (Ledergerber *et al.* 1999; DeGruttola *et al.* 2000; FHI and CAPRISA 2010; Varghese *et al.* 2010). This is the consequence of possible mutations that emerge in the viral proteins embattled by antiretroviral agents, or the virus undergoes a transition to a latent phase and stop producing the viral proteins targeted by the drugs (Cohen 2002). As a consequence of such natural resistance against antiretroviral drugs by HIV, it is really a matter of distress that the patients suffering from AIDS, at present, have no hope for a promising treatment. In this context, a new alternative therapeutic approach is badly needed which can be effective at the gene level, targeting and deactivating the gene responsible for coding the genetic material of HIV (Vandekerckhove *et al.* 2006). The use of RNAi might prove to be an effective alternative to HAATR (Capodici *et al.* 2002).

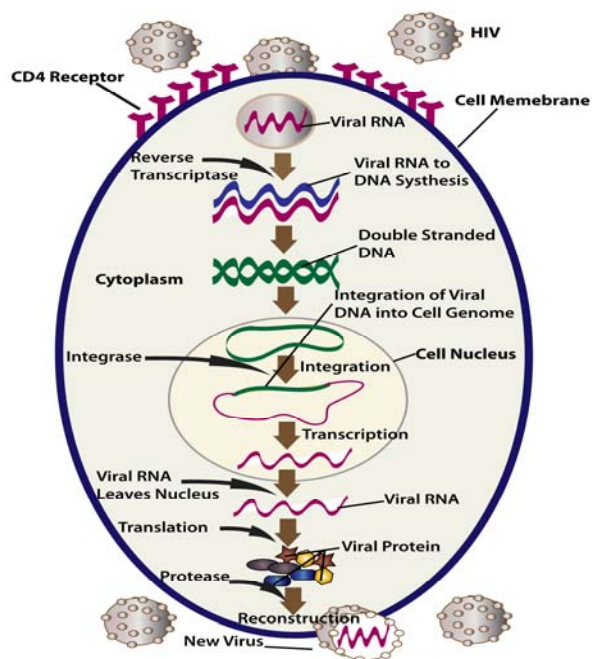


Fig. 4. The replication cycle of HIV

Albeit there is no effective vaccine for the treatment of HIV/AIDS, yet the only treatment available to the patients is the post viral exposure prophylaxis. Presently, the most available treatment option for the disease is HAART. As discussed above, the two main types of drugs which are frequently used for treatment are the reverse transcriptase inhibitor (RTI) and the protease inhibitor (PI). These are meant to diminish the potency of HIV infection immediately after exposure (Patrick *et al.* 2004). The RTI drugs potentially act by interfering with the reverse transcription of the viral genome, where the viral RNA is converted by the reverse transcriptase into the viral DNA (Moatti *et al.*, 2003). The RTI drugs are again of two main types *viz.*, nucleotide RTI and non-nucleotide RTI. The basic thing behind the first one is that it is made up of flawed nucleotide sequences which are implanted by the reverse transcriptase into the newly synthesized viral DNA. As a consequence, both budding and replication of viral DNA come to a halt (Dybul *et al.* 2002; Hirschel *et al.* 2008). However, the major drawback of this drug is that when this drug is administered at high dose, it becomes very toxic to the patient. The doctors therefore prescribe this drug at low dose, which is not enough to abolish the higher number of viral particles inside the host. The second drug (non-nucleotide RTI) is some sort of protein that functions on the active site of the reverse transcriptase and makes some conformational changes there. Due to changes in

the structure of its chemical foundation, the enzyme is not able to convert the viral RNA to the DNA. As the total genetic information of HIV is confined to its RNA, it cannot be integrated into the host cells owing to the drug action. Accordingly, the HIV cannot lead the host cells to synthesize virion particles and thus prevent the replication process (Blankson *et al.* 2002; Cohen 2002). The principal drawback of these drugs is that they cannot cope up with the extremely high level of mutation that occurs during reverse transcription as it is a very much error-prone process. As a result of this, the genes coding for reverse transcriptase mutate very often and the mutated virus is capable of producing very much structurally altered reverse transcriptase. Consequently, the active site of the enzyme becomes unrecognizable and it cannot be inactivated by the drugs (Martinez-Picado *et al.* 2000).

The main principle of the PI drugs, on the other hand, is to interfere with the HIV protease, which is necessary to activate the inactive viral structural proteins (*Gag* and *Pol*) that need to be cleaved to produce different viral particles required for replication. The binding of PI drugs to the active site of the inactive *Gag* and *Pol* leads to complete disruption of the viral replication. As a consequence, HIV is unable to produce its new copies and thus cannot destroy host cells. The major drawback of this drug is just like the non-nucleotide RTI drugs where the mutated gene encoding for the protease cannot be recognized due to the high rate of mutation (Blankson *et al.* 2002; Cohen 2002).

However, very recently an industry-academic collaboration in California, with financial support from the California Institute for Regenerative Medicine, has yielded a convenient gene therapy strategy for blocking HIV infection. A team of scientists is now preparing a clinical trial in AIDS patients with lymphoma who are prime candidates for the hematopoietic stem cell (HSCs) transplantation, which is a key part of the technique. The method uses a zinc finger nuclease to create HSCs, missing both copies of the *CC chemokine receptor 5* (*CCR5*; *CD195*) gene. Most HIV strains cannot colonize cells without *CCR5*, so the genetic modification deprives the virus of a place to bind and breed (Holt *et al.* 2010; Osherovich, 2010). On the other hand, Benitec Ltd. in Australia also developed a lentivirus-based therapy for HSCs. It combines a *CCR5*-disrupting ribozyme with small hairpin RNA against the HIV reverse transcriptase gene and the trans-activation response (TAR) region of the HIV genome. This is still an unnamed therapy which is in a Phase I trial to treat HIV/AIDS in lymphoma patients (DiGiusto *et al.* 2010; Osherovich 2010).

Therapeutic applications of RNAi in HIV/AIDS treatment

There are two components involved in the therapeutic applications of RNAi. These are siRNA and miRNA, of which the first one is thought to be the main protagonists in the RNA interference process (Berkhout and Brake 2009). Several groups of scientists have shown that siRNA can restrain HIV replication efficiently in cell/tissue cultures. HIV infection can be blocked by targeting either viral genes (*Gag*, *Rev*, *Tat* and *Env*), or human genes (for example, *CD4*), the principal receptor of HIV that are involved in the HIV replication cycle. Studies showed that siRNA is able to restrain HIV-1 infection drastically (Rana 2007). This is promising, as antiviral therapies that can attack multiple viral and cellular targets could circumvent genetic resistance of HIV.

There are two types of HIV, HIV-1 and HIV-2 that affect human cells. These are distinguished based on their packaging to the RNA. HIV-1 is able to bind with any appropriate RNA, while HIV-2 is privileged to bind with the mRNA. While similar in many ways, there are important differences between HIV-1 and HIV-2 that provide insights into virus evolution, tropism and pathogenesis. Major differences include reduced pathogenicity of HIV-2 relative to HIV-1, enhanced immune control of HIV-2 infection and often some degree of CD4-independence. For this reason, HIV-1 is much more efficient to mutate with higher frequency. So, HIV-1 infection proceeds to AIDS faster than HIV-2 infection and is liable to the mass number of global infections (Reeves and Doms 2002). In order to accomplish effective inhibition of HIV-1 replication *in vivo* by using RNAi pathway, one should concentrate on the following: (i) the cells to be targeted, (ii) the viral vector to be used, (iii) the HIV-1 genome or the cellular co-factors important for HIV-1 replication, and (iv) the risk of HIV-1 escaping from the RNAi strategy (Vandekerckhove *et al.* 2006).

The principal host cell for HIV infection comes either from lymphoid (*e.g.* $CD4^+$ lymphocytes) or from myeloid (*e.g.* macrophage, microglia, monocytes or dendritic cells) source of origin. The best effective way is to choose the $CD4^+$ lymphocytes for the successful gene therapy for AIDS as the $CD4^+$ cells can easily be isolated, transduced, expanded and selected by culturing *in vitro*, though it has a major disadvantage in having low life expectancy (Vandekerckhove *et al.* 2006). However, this approach potentially has utility as a protective 'therapeutic vaccine' of virus-susceptible lymphocytes. Particular attention is given to advances in

combinatorial gene expression systems that prevent the emergence of RNAi-resistant virus by simultaneously targeting multiple HIV targets (Barichievy *et al.* 2009).

In dealing with siRNA-based therapy, the major concern is how efficiently the siRNA can be transfected in the target cells without any degradation of its component. Here, the effective and functional way of delivery of siRNA to the target cells would be to use the lentiviral vectors and RNA packaged in micelles. Nowadays, lentiviral vector constructs have proven to be very productive in terms of transduction due to their ability to infect both replicating and non-replicating cells, including stem cells and, therefore, becoming the vectors of choice for siRNA delivery (Sachdeva *et al.* 2007). The Lentiviruses are a type of retrovirus that can get through the intact membrane of the nucleus of the target cell with the help of their pre-integration complex (virus "shell"). The vector can be made by cloning a reporter gene or therapeutic gene into a vector sequence that is flanked by LTRs (necessary for integration of the therapeutic gene into the genome), and the Psi-sequence (acts as a signal sequence and is necessary for packaging RNA with the reporter or therapeutic gene) of HIV. The use of certain lentiviral vectors is absolutely fascinating, especially when it is considered how it will contribute to prevent HIV virions from being produced and released from the infected cells (Morris and Rossi 2006).

Most recently, another novel vector-based therapy for the treatment of individuals infected with HIV-1 is under investigation. This is named as SV40-based therapy. The SV40 vectors are also intrinsically safe and they can transfect both non-dividing and dividing cells. This therapy involves the application of RNAi to prevent infection of new cells with HIV-1 and therefore, eventually cure infection. An SV40-based vector will be used to transfer the therapeutic anti-HIV-1 sequence to T-cells of HIV-1 infected individuals in order to build up long-lasting improvements of their condition (HIVSTOP 2010).

Unfortunately, there is an impediment in the treatment of AIDS *i.e.* HIV virus can flee from RNAi pathway through base mutations. The virus also keeps away from being damaged by using alternative splicing to try to avoid being embattled *via* RNAi (Boden *et al.* 2003; Das *et al.* 2004; Westerhout *et al.* 2005). So nowadays, scientists put much more effort to create multiple siRNAs targeting conserved nucleotide sequence of the HIV-1 that are much less vulnerable to mutations such as the promoter sequence of HIV-1. The HIV-1 virus ensures that very little mutation is occurred in those conserved regions (Morris and Rossi 2006).

Limitations of the RNAi therapy

Although the RNAi therapy at present holds a great promise in treating AIDS, yet it has some limitations like all other therapies. There are mainly three possible limitations that can impede the successful treatment of HIV. Firstly, for successful gene silencing siRNA is constructed to cleave RNA of specific sequence in the RNAi pathway. If it does not happen, it leads to the silencing of non-targeted gene which can induce unforeseen effect. Secondly, a lot of efforts have been made for the delivery of siRNA in the host cells for successful silencing of the target genes. The major advancement in this process is the discovery of lentiviral vectors. However, an efficient delivery system is still sought for other viral diseases. Thirdly, point mutation and alternative splicing within the target gene can also lead to the failure of RNAi therapy (Berkhout 2010).

Concluding remarks

The relative ease with which genes can be silenced using RNAi has caused a minor revolution in molecular biology. Armed with data from genome sequencing projects, gene silencing with RNAi can be used on a breathtaking scale. As RNAi relies on the sequence-specific interaction between siRNA and mRNA, siRNAs can be tailored to silence almost any gene. siRNAs that have been chemically synthesized or created by *in vitro* transcription systems can induce silencing in several systems, including mammalian cells. Therefore, apart from the current treatment facilities of RTI and PI drugs for HIV/AIDS, the siRNA based therapy holds certain advantages that make it a promising treatment option. siRNAs are easy to construct and relatively cost efficient. They can be packaged in lentiviral vectors and delivered into the target cells. In addition, the use of siRNA, unlike protease inhibitor drugs can employ strategies designed to overcome a key obstacle to the efficacy of protease and reverse transcriptase inhibitor drugs. To overcome the resistance inferred by HIV-1 viruses through their ability to mutate target sites, scientists believe that siRNAs can be constructed for targeting the conserved regions. A key disadvantage to using RNAi as a treatment is the possibility of inadvertent of targeted gene silencing *in vivo*. However, more research in this field is needed to overcome these limitations. Even though RNAi treatment appears to be an effective tool in the inhibition and cure of the HIV/AIDS, in the future it is probable to be used in combination with other treatment options to generate a very potential, dominant and highly active antiretroviral treatment concoction.

In the end, it can be said that information obtained from ongoing and future experimental studies using RNAi will give a much greater insight on how to utilize this rapidly developing genetic tool to effectively fight viral related diseases. Until the scientists uncover ways to resolve some of the risks linked with RNAi therapy, particularly with siRNA, it is best to remain cautious rather than excessively optimistic in the evaluation of this therapeutic approach.

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