Modern biotechnology-based therapeutic approaches against HIV infection (Review)

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Received August 18, 2017; Accepted September 27, 2017

DOI: 10.3892/br.2017.1006

Abstract. The causative agent of acquired immune deficiency syndrome (AIDS) is human immunodeficiency virus (HIV). Since its discovery before 30 years, a number of drugs known as highly active antiretroviral therapy have been developed to suppress the life cycle of the virus at different stages. With the current therapeutic approaches, ending AIDS means providing treatment to 35 million individuals living with HIV for the rest of their lives or until a cure is developed. Additionally, therapy is associated with various other challenges such as potential of drug resistance, toxicity and presence of latent viral reservoir. Therefore, it is imperative to search for treatments and to identify new therapeutic approaches against HIV infection to avoid daily intake of drugs. The aim of the current review was to summarize different therapeutic strategies against HIV infection, including stem cell therapy, RNA interference, CRISPR/Cas9 pathways, antibodies, intrabodies and nanotechnology. Silencing RNA against chemokine receptor 5 and other HIV RNAs have been tested and found to elicit homology-based, post-transcriptional silencing. The CRISPR/Cas9 is a gene editing technology that produces a double-stranded nick in the virus DNA, which is repaired by the host machinery either by non-homology end joining mechanism or via homology recombination leading to insertion, deletion mutation which further leads to frame shift mutation and non-functional products. Intrabodies are intracellular-expressed antibodies that are directed towards the targets inside the cell unlike the naturally expressed antibodies which target outside the cell. Different nanotechnology-based therapeutic approaches are also in progress against HIV. HIV eradication is not feasible without deploying a cure or vaccine alongside the treatment.

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Key words: antibodies, CRISPR/Cas9, drugs, HIV, nanomedicine, RNAi, stem cell, therapy

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1. Introduction

Acquired immunodeficiency syndrome (AIDS) was identified in 1981 (1). Subsequently, it was found that the causative agent of AIDS was HIV. Currently, over 35 million individuals are HIV infected worldwide with the highest prevalence being in Sub-Saharan African countries (2,3). HIV type 1 is the most prevalent type of this virus (4). Since the year 2000, there has been a reduction in the number of new cases of HIV infection from 3.1 to 2 million globally (3). Moreover, the number of HIV infected subjects on antiretroviral therapy has increased from 1 to 15 million. The main reason behind this achievement was raising the fund against HIV infection from $4.9 to $21.7 billion (5). Thus, throughout there is struggling to manage HIV infection and to a greater extent there has been

success in achieving this target. Nevertheless, the eradication of HIV remains a target.

Human immunodeficiency virus attacks the immune system and leads to the development of AIDS. The natural history of HIV infection is diverse. The average duration required for infection to reach the stage of AIDS is 8-10 years. Approximately 5-10% of HIV-exposed subjects remain asymptomatic even though they do not take any retroviral drugs, and are known as long-term non-progressors (6,7). The presence of such groups of HIV-infected subjects highlights the fact that there is natural resistance to the virus. The exploration of the host natural resistance against the virus may lead to the development of novel therapeutic approaches.

Drug development against HIV infection is a challenging task. HIV is a retrovirus with high variability in its genome due to a lack of proofreading exo-nuclease activity in its reverse transcriptase enzyme. The high genome variability of HIV genome in a patient results in the production of quasi-species and drug resistance against previously effective drugs (8). Therefore, researchers are seeking better therapeutic approaches against HIV infection. These approaches are discussed below.

2. Stem cell therapy

The first step of HIV life cycle is its binding and entry into the host cell. The HIV envelop protein (Env) binds with its receptor and co-receptor on the target cell. The HIV Env protein is a heavily glycosylated trimer of gp120 and gp41 proteins. CD4 is a member of the immunoglobulin superfamily and acts as a receptor, while chemokine receptor (CCR) 5 or chemokine X receptor 4 (CXCR4) acts as a co-receptor for viral entry into the host cell. The viruses that exploit CCR5 or CXCR4 are termed R5 and X4 HIV virions, respectively, while those using both CCR5 and CXCR4 as co-receptors are known as R5X4 HIV virions (9,10). However, HIV viruses that are transmitted by sexual contact, percutaneous inoculation or maternal routes, are R5 viruses (11). Thus CCR5 plays an important role in HIV entrance into the host cell. The deletion mutation, Δ 32 CCR5 results in the production of defective protein. The homozygous condition of Δ 32 CCR5 prevents HIV infection as the virus cannot enter the host cell. Furthermore, the stem cell therapy from a homozygous Δ 32 CCR5 deletion mutated donor to the HIV-infected patients demonstrated a successful therapeutic option (12-14). However, the duplication of this experiment was failed in six other patients highlighting that this therapeutic approach is extremely difficult (13). Furthermore, the problem with allogeneic stem-cell transplantation from an HLA-matched donor, is the low availability of donors and high risk associated with allogeneic stem-cell transplantation.

Another approach of gene therapy is to permanently disable CCR5 by zinc finger nuclease (NCT00842634). Zinc finger nucleases are genomic scissors comprising DNA binding and cleaving domains (15). The main limitation of this genome editing technology is the overlapping of individual zinc finger specificity. In a clinical trial commencing in 2014, SB-728mR-T treatment was delivered using many *ex vivo* adenovirus expanded transduced autologous $CD4^+$ T cells into 12 HIV-infected patients. No severe side effects of transduction were reported. For substantial effects of transduction, biallelic transduction of CCR5 is required. However, in the majority of circulating autologous $CD4^+$ T cases, there was knockdown of one CCR5 allele. The study is currently ongoing (NCT00842634) and is to be completed in June, 2018 (16).

3. RNAi

Preclinical studies have shown that small interfering RNAs are less immunogenic than protein-based agents and potent inhibitors (14). Silencing RNA (siRNAs) against CCR5 (17,18) and other HIV RNAs (19-22) have been tested and found to elicit homology-based, post-transcriptional silencing. Taken together, it was concluded that the efficacy of this technique is highly dependent on various factors including the use of vectors and promoters for the expression of siRNAs and selection cassette combinations for controlling HIV infection (23).

4. CRISPR/Cas9

The most previously used gene editing technology for targeting HIV infection is clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein-9 nuclease (Cas9). The technology comprises CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracr RNA). crRNA and tracr RNA are collectively known as guide RNA (gRNA) which directs caspase-9 to the target site (24). The target site is 20 nucleotide DNA sequences, which is complementary to crRNA and is followed by protospacer adjacent motif (PAM) of 3 nucleotides (NGG). Caspase-9 produces double-stranded nick just before PAM sequence. The double-stranded nicks produced in DNA are repaired by host machinery via non-homology end joining mechanism or homologous recombination leading to insertion, and deletion mutation, which further leads to frame shift mutation and non-functional products (25). To avoid off-target sites of this technology alternation in caspase-9 nuclease is induced, resulting in the production of single-strand nick in DNA (26). The repair mechanism requires two target sites in close proximity and the likelihood of off-target sites are substantially reduced. The technology is limited to only those target sequences that are 20 nucleotides in length and followed by PAM. Moreover, certain target sequences are problematic due to the formation of secondary RNA structures. Most previously engineered RNA-guided FokI-nucleases have been used to improve the cleavage efficacy and broader genome targetibility (27).

Previous studies of 1-cell mouse embryos demonstrated that the cytoplasmic microinjection of gRNA and CAS9 mRNA to generate enhancer knockout mouse lines possessed a range of putative off-target sites (28). However, sequencing of amplified products showed that there were no off-target effects in the genomes (28). The study results suggested that the potential off-target effects of CRISPR/CAS9 technology are exaggerated. Thus, this technology is highly effective and accurate for deleting putative gene enhancer sequences from the mouse genome (28).

5. Antibody-based therapeutic approaches against HIV infection

Antibody-based therapeutic approaches against HIV infection have had limited success thus far. The main reason behind

this therapeutic approach is its transient effect and specificity. Recently recombinant adenoassociated virus (rAAV) vector is used as a delivery mechanism of broadly neutralizing (bnAbs) anti-HIV antibodies in monkeys and mice and it demonstrated long-lasting immune responses (29). The genetically modified rAAV persists in the cell and only produces the gene of interest for the entire life of cell (30,31). However, some unwanted immune responses which limit the efficacy of these bnAbs have also been identified (29).

6. Antibodies inside the cell

Intrabodies are intracellular-expressed antibodies that are directed towards the targets inside the cell unlike the naturally expressed antibodies which target the outside of the cell. An intrabody approach functions with single antigen binding fragment or may even contain a single domain (nanobodies). However, the cytosolic expression of intrabodies may lead to the production of non-functional antibodies due to misfolding (32,33). To overcome the problem, such intrabodies are produced that are, not only specific towards their targets (functional knockdown of membrane protein or some other protein), but may also possess an endoplasmic reticulum (ER) retention signal, the amino acid signal (34). Thus, the intrabody is retained with its target inside the ER (34).

7. Nanotechnology against HIV infection

Nanotechnology is an emerging field of science and technology that is revolutionizing the medical field. Typically, nanoparticles range from 1 to 100 nm in size in at least one dimension (35). Nanoparticles are more commonly referred to as nanomedicines and are used for the prevention and diagnosis of infections (36). In some cases, the healing and therapeutic potential of nanomedicines have also been reported (37,38). Most of its applications are reported in the field of cancer. Multiplte nanosystems are either Food and Drugs Authority approved or in clinical trials for the treatment of systematic cancer (38,39). The success of nanomedicines are attributed to improved delivery of poor water soluble drugs, targeted delivery to cells or tissues, intracellular delivery of macromolecules and controlled release of drug at its target site (35,39,40).

Nanomedicines against HIV infection have also been previously investigated. Experiments on mice have shown that nanosuspension of indinavir (a retroviral drug against HIV infection) was stabilized by a surfactant system for effective delivery to various tissues (40,41). The nanosuspension of indinavir was loaded on macrophages and its uptake was identified in different tissues including spleen, liver, lungs and brain. Moreover, the half life of conventionally delivered indinavir in rodents was 2 h, while a single dose of intravenously injected nanoindavir suspension in rodents was measurable in the blood up to 14 days post-treatment (41). It was observed that the cellular uptake of satuvudine (HIV nucleoside analog reverse transcriptase inhibitor) encapsulated in various liposomes and conjugated with mannose and galactose was also increased as compared to plain liposomes or free drugs (41,42).

In addition to delivery agents, nanomaterials as therapeutic agents have also been reported. It has been shown that the capsid structure of HIV may be used as a target for structure-based drugs to inhibit viral replication (43,44). The *in vitro* anti-HIV activity of various fullerene-based structures including dendrimers and inorganic particles such as gold and silver have already been previously reported (45-47).

8. Conclusions

The only available solution of HIV-infected subjects is highly active antiretroviral therapy and the drugs are used in combination to suppress the virus at any stage of its life cycle. These drugs only increase the life span of the subjects and do not provide a permanent cure. Therefore, different novel therapeutic approaches against HIV, which include stem cell therapy, genome editing, antibodies, and nanotechnology are under investigation. Among these technologies, CRISPR/Cas9, genome editing technology seems to be the most potential therapeutic approach against HIV infection.

Acknowledgements

The present study was supported by Department of Microbiology, University of Health Sciences and Faculty of Health.

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