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A novel antiviral approach

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ABSTRACT

Viral infections are often the etiological agents of severe acute and chronic human diseases. Their peculiar biology usually leads to the need of design specific therapies for each virus, and the eradication of the viruses and the healing of the patients very often are not reached also after decades of theoretical and applied researches. HIV is a classical example of how the efforts of the researchers may be disappointed in eradicating a virus infection in an infected patient. Here I present a hypothesis for a new antiviral approach that may be suitable for the treatment of HIV infected patients. The same approach, with opportune modifications, may be also applied as healing strategy for a wide set of viruses infections. In brief, my idea is to use the retrotranscription machinery and the packaging system of HIV infected cells to amplify the interfering effects of siRNAs directed against HIV genes and transcripts. The coding sequences for the interfering RNAs are brought to the infected cells via modified HIV virions deficient for structural viral genes that will use the resident viral activities of HIV infected cell as helpers. The use of this strategy will probably lead to an intracellular, intercellular and systemic amplification of the specific virus-targeted interfering activities. Moreover this strategy may show novel levels of interference: a competition between the deficient and wild type viruses for the packaging molecules and the possibility of homologous recombination between the deficient and wild type viruses that may lead in turn to the formation of recombinant non infectious viruses, and the removing of wild type provirus sequence from the host genome of infected cells by recombination.

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Introduction

Since the discovery of HIV, several approaches have been attempted to control or eradicate the HIV infection. Strategies span from antiretroviral approaches, drug targeted therapies, aptamers, gene therapy on precursor cells, HIV-specific siRNAs, ribozymes and many others. Unluckily, no efficient way to eradicate HIV from infected individuals has been still developed [1].

The most common strategy used nowadays is a drug cocktail that specifically inhibits several key steps of the viral life cycle in a therapeutic regimen called HAART. Unluckily, HAART treated patients usually have to follow the protocol for their entire life with no eradication of the disease, severe chronic toxic effects and high expenses either for the community or for the patient [1].

The need to develop a very efficient way to control or eradicate HIV is still urgent. It is true that many steps have been taken towards a therapy, but HIV still is a step ahead of the researches. One of the reasons of this phenomenon is the low fidelity of HIV reverse transcriptase that allows HIV to evolve at a very high rate [1].

Several strategies take advantage from RNA interference mediated gene silencing or translating inhibition operated by siRNAs and miRNAs and related molecules. RNA mediated interference has key regulatory functions in many cellular processes, such as differentiation, development, and metabolism [2–4], but also during virus infection. The possibility of using multiple siRNAs to interfere and inhibit specific HIV functions has many charming characteristics, first of all the possibility to bypass the capacity of HIV to escape from therapies taking advantage from its high evolutionary rate. This is why many efforts are taken from the researchers to design efficient HIV targeted siRNAs and, most of all, to design efficient vectors for the delivery of molecules specifically to infected or infectable cells, to design respectively a healing or a protective strategy [5].

HIV, and other lentiviruses, have also had a great impact in research thanks to their unique features. HIV derived vectors are used commonly as very efficient transgenes vectors. They show high rate of gene transfer and the capacity of integration into the host genome (a key characteristic of retroviral vectors), differently from episomial viruses based vectors [6]; moreover they show the ability to establish a long lasting transgene expression. Lentiviruses derived vectors also show a bias in the integration on introns of highly expressed genes, avoiding the oncogenic potential of the promoter biased vectors. Another big advantage of lentivirus derived vectors is their ability to infect both dividing and not dividing cells and potentially a wide spectrum of cell type, due to the vector design. Finally, HIV derived vectors usually can carry up to 7 Kbs or more without losing their infectious capacity. For a very good

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review about HIV replication cycle and HIV derived vectors please refer to Pluta et Kacprzac [7].

RNA interference (RNAi) is now considered as a powerful tool to negatively regulate gene expression in almost any eukarya. Its pathway is still under intense study and details of its biological and molecular mechanism of action have been described elsewhere [8,9]. In brief, long dsRNA (double stranded RNA) molecules are cleaved by the endonuclease Dicer into short 20–30 (with a bias of 21–25) nucleotide small interfering RNAs (siRNAs) which are in turn incorporated into a RISC (RNA induced silencing complex). RISC is a multi-proteic complex that selectively degrades mRNAs that share sequence homology with the siRNA loaded in the RISC, with a bias with the sequence homology in the 3'UTR of the transcript, even if this is not a rule. In mammalian systems, and so in humans, siRNAs can be delivered *per se* externally using several strategies, such as RNA electroporation, or expressed endogenously as long precursors (to be later cleaved) from pol III promoters (these precursors are called short hairpin RNAs or shRNAs) or from pol II promoters (as long precursors that will be cleaved into micro RNAs or miRNAs, or miRs), that will result into specific downregulation of target mRNAs [10–13] and sometimes into the silencing of genomic loci that share sequence homology with the interfering RNA molecules.

The hypothesis

The strategy here suggested is to use in vitro assembled HIV derived lentivirus vectors to deliver *in vivo* specific anti-HIV molecules; specifically here I suggest to use vectors able to induce the transcription of shRNAs and the generation of siRNAs against HIV specific transcripts.

These in vitro assembled vectors must be deficient for structural viral genes (e.g. *gag*, *pol* and *env*) but must retain the recognition sequences to be retrotranscribed, packed (LTRs and Psi sequences), and to have the transducted sequences transcribed. Many commercial vectors share these characteristics [14].

From now on, the so designed vectors will be called H-Virions when packed, H-Vectors when I will refer to the RNA vectors, and H-proviruses when I will refer to the retrotranscribed and integrated vector. "H" is for Healing. Wt-(Wild type) Virions, Viruses, Proviruses, transcript et cetera will be used to indicate respectively infective HIV Virions, Viruses, Proviruses, transcript et cetera that can be found in the population.

The shRNA sequences transducted by H-Vectors and related siRNAs are to be designed to target specific wt-HIV genes and transcripts, such as *Gag*, *Pol*, *Env* or *Tat, Rev* and *Nef* genes and their transcripts; a very good review about possible shRNAs with these characteristics can be found in Mcintyre et al. [15]. Specifically, it seems that the early transcribed *Tat, Rev* and *Nef* genes [16] could be good candidates to be used to interfere with HIV infection [17].

The use of this strategy could lead to several advantages when used *in vivo* in wt-HIV infected patients.

1. The H-Virions will infect the same cells that are potential targets of wt-HIV virions. There will be no H-Virions internalization in cells that are not potentially targeted by wt-HIV virions.
2. Several strategies to target specifically HIV-infected cells in the contest of an infected organism have been described elsewhere [18–21]; the possibility to target the H-Virions or the H-Vectors specifically towards HIV-infected cells may help to enhance the safety of the approach here described.
3. If the H-Virion infects a cell that is not a wt-HIV infected cell, the vector will be reasonably retained for a certain degree of time inside the cell and, due to a possible latter infection from wt-HIV, will be activated and assert its functions, conferring a mild protective activity against a productive wt-HIV infection.
4. Instead, if the sequences born by the H-Vector will be retrotranscribed and integrated into the host genome by the enzymatic activities brought inside the H-Virions, the protecting activity could be even greater.
5. Instead, if the H-Virion infects a cell that is yet been infected by a wt-HIV, the wt-HIV will function as a perfect helper for the healing capacity of H-virions. These cells will be called co-infected cells (CICs). Specific targeting of H-Virions towards HIV-infected cells [18–21] may help to increase the CICs numbers after administration, enhancing efficacy and safety.
6. In CICs, H-proviruses will actively transcribe shRNAs, and the wt-HIV specific siRNAs will lead to a decreased activity of wt-HIV functions and infectivity. The capacity to design H-vectors able to produce multiply siRNAs could lead to an interfering activity against several wt HIV transcripts, broadening the capacity of H-Vectors to interfere with wt-HIV functions. Moreover, due to the multiple siRNAs production and their short length, H-Vectors could easily avoid the escaping mechanisms of wt-HIV thanks to the high error rate of retrotransciptase and the subsequent high rate of mutations. H-Vectors could easily target many wt-HIV variants, also those newly generated in the patient during the wt-HIV infection.
7. In CICs, the H-vectors will interfere with wt-HIV acting as quencher of wt-HIV targeted machineries, lowering the activity of wt-HIV.
8. In CICs, H-vectors will interfere with wt-HIV packaging system, so a certain degree of virions produced by CICs will be H-Virions instead of wt-HIV virions.
9. There is the possibility that in CICs, H-Provirus and wt-HIV provirus could recombine by homologous recombination leading to the disruption of wt-HIV provirus and thus either eradicate the infection from those cells or leading to chromosomal rearrangements that could lead to cell death.
10. There is the possibility that in CICs, H-Provirus and wt-HIV provirus could recombine, leading to the creation of deficiency viruses, not able to induce an effective wt-HIV infection but still retaining part of the siRNA activity and thus maintaining the protective function. Even if some infective HIV virions could be produced by these events of recombination, probably they will act as self-limiting viruses because they would have incorporated self-targeting siRNAs.
11. Remarkably, the H-Virions produced by CICs could amplify intercellulary and systemically the spreading of H-virions and their healing and protective activity. So, theoretically, just a single dose treatment could lead to the protective, therapeutic and possibly healing capacity of H-Virions.
12. H-Virions will share many surface epitopes with wt-Virions, so they may enhance the normal host immune response against the pathogen.

Evaluation of the hypothesis

The use of HIV derived lentivirus vectors as an effective way to transfer genetic material and as tool in research on mammal systems are established data. Many HIV derived lentivirus vectors are now commercially available and are used in many laboratory routine techniques as routine expression vectors [14]. The innovative proposal of the present hypothesis is to transfer the knowledge and expertise from the laboratory to the therapy; to transform a research tool to a healing strategy to be used *in vivo* in patients. The use of shRNAs and siRNAs to modulate the transcriptome output

has also been widely analyzed and used in mammal systems. The use of shRNAs and siRNAs as therapeutic effectors has been widely postulated and partially used in many human diseases, and HIV makes no exception [15]. The innovative approach of the present hypothesis is to combine the siRNA technology with viral vectors derived from the same disease that is to be cured, assuring host cell specificity, many degrees of interference with viral infection both intracellularly and systemically, as an amplification of the healing molecules intracellularly and of the healing vectors systemically, and a possible protection for non infected cells. Moreover, in theory, a one dose or few doses treatment with almost no side effects would be effective. It should be tested if the sequences born by the H-Vectors should be put under the control of viral promoters (to enhance the interfering capacity of the H-Vectors and the specificity of the response in CICs) or host promoters (to enhance the protective effect in non wt-HIV infected cells, but with the risk to increase potential side effects). *In vivo* and in vitro data are needed to confirm or confute the hypothesis, but in the belief of the author the hypothesis is worth to be investigated.

Discussion

The hypothesis is about the combination of accepted molecular mechanisms and used technologies, bringing a research tool such as HIV-derived lentiviral vectors into therapy. The prediction of the impact of the therapy here described is difficult to formulate, but reasonably it could lead from an attenuation of the symptoms to a complete eradication of the disease. Of course the variables are many, from the serotype of the patient, the personal response to the therapy, the stage of the disease at which the therapy is administrated, to the specific design of the vector and the previous treatments born by the patients, especially the antiretroviral therapies now broadly uses in the HAART cocktail.

Interestingly, the strategy here presented could be combined with immunotherapeutic strategies, where stimulated T cells are targeted to infected cells with the aim to eradicate the infection [17,22]. The strategy here described could limit the HIV spread, while via immunotherapeutic strategies infected cells could be eradicated. Please note that while CICs should have their ability to produce infective virions severely impaired or even stopped, they can be still recognized by the immune system as infected cells.

Another potential risk of a so designed therapy may reside in the possibility of transferring the H-Virions from a treated patient to another person by unprotected sexual intercourse or blood contamination, as it may happen in the habit of sharing needles in endovenous drug administration in many drug-addicted communities. I really think that the risk to share H-Virions together with infective wt-HIV virions is a well balanced risk, that in theory may also have an epidemiologically positive effect into the possibility of eradicate the HIV infection from the population, especially in areas where traditional administration of therapy may result difficult. Of course this approach raises serious ethical considerations. Another weak point of the theory is that a so designed treatment could lead to a co-evolution of the H- and wt-Virions inside the patients, but the use of multiple shRNAs in the H-Vectors should minimize the related risks.

Advantages of the method could of course be great, from the relatively easiness of the massive production of H-Vectors, to the possibility of one or few dose therapy approach, to the possibility to design specific H-Vectors *ad hoc* for each serotype, to the possibility to administrate a cocktail of several H-Vectors to the same patient and so on; the H-Vector strategy seems to be potentially a very flexible tool.

If the hypothesis will be confirmed, the H-Vectors should work both intracellularly and systemically. H-Vectors should protect non infected cells from further infection and in CICs they should interfere with HIV functions at many levels: they should stop or lower the production of viral factors via siRNAs and drain the remaining viral factors for their own metabolism, acting as a "virus of the virus". So the infected cells should survive longer and the non infected cells should have a sort of protection from the infection. Moreover, the CICs where the RNA interference is not able to stop the production of viral factors, will produce together with the wt-Virions also H-Virions, allowing a systemic spread of the H-Vectors with their healing and/or protective activities. Moreover, the ratio between infective vs non infective virions should decrease, allowing the residual immune system of the host to built up a more efficient immune response to the pathogen, maybe in combination with an immunotherapeutic strategy, as described. In the best scenario the H-Strategy should be able to eradicate the infection, in a milder scenario, the H-Strategy should stop the progression of the disease, improve the patients' conditions with almost no side effects.

If the strategy here described should be effective as expected, the same rationale might be used as healing strategy or in combination with other approaches for several diseases of viral origin that still escape traditional therapies, such as the widespread HCV.

The use of virus-X derived deficiency vectors able to produce specific siRNAs against key components of the virus-X metabolism might became in the future a new antiviral approach, because these healing viruses will have the same host spectrum, will specifically interfere with the virus functions in the co-infected cells and may manifest a protective effect in non infected cells.

Conflict of interest statement

None declared.

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