Promoter targeted small RNAs: stabilising viral reservoirs

The viral reservoir, a major barrier to curative therapy for HIV-1 infection, consists of viral DNA sequestered in long-lived resting CD4+ T cells and macrophages in diverse anatomical sites. Some of these sites are recognised parts of the immune system, such as lymph nodes. Others, such as the brain are not. Current anti-retroviral therapy (ART) does not substantially impact upon this reservoir. Early intervention with ART limits the reservoir but does not result in its elimination

This reservoir is believed to consist of integrated virus, which is in a latent state, silenced by epigenetic modifications of histone tails in the nucleosomes associated within the viral promoter, the 5’LTR. These histone tail modifications, which include deacetylation and methylation of lysines 9 and 27 of histone 3 (H3K9 and H3K27), are induced by the recruitment of enzymes such as histone deacetylases (HDACs) and histone methyltransferases.

Most current efforts aimed at modulating the reservoir have attempted to reverse this silencing. However, trials of global activation of T cells by cytokine stimulation or T cell receptor stimulation have failed. An alternate more recent approach has been to reverse latency via HDAC inhibitors such as valproate, vironostat and panabinostat. These have induced modest increases in viral transcript levels and are being explored. Therefore, although this approach is likely limited by non-specific gene activation and by the fact that viral latency is determined by complex epigenetic machinery of which HDACs are only one component.

An alternate, far less explored, approach is to stabilise the reservoir by enforcing viral latency. This can be achieved by short interfering (si) or short hairpin (sh)RNAs targeting the 5’LTR of HIV-1. These short double stranded RNAs have the ability to induce transcriptional gene silencing (TGS) of the virus (Figure 1). The virus is unable to produce any mRNA transcripts from its genome and therefore cannot replicate. TGS, as distinct from siRNA induced post-transcriptional gene silencing of HIV-1, is long lasting and relatively resistant to escape. It produces far greater and more sustained decreases in viral load than the most promising PTGS inducing siRNAs. A single dose of siRNAs targeting the highly conserved, unique sequences of the tandem repeat of the NF-κB binding motif, which is located within the non-transcribed portion of 5’LTR, completely silences HIV-1 replication in vitro for >30 days. When delivered by retrovirus, as a shRNA, it silences the virus for >1 year in vitro. The effect of these constructs is highly specific. They do not interfere with cell growth or viability nor do they change the expression of host genes regulated by the transcription factor NF-κB, nor do they induce an inflammatory response through induction of interferons.

These constructs turn off viral transcription by inducing and then enforcing epigenetic changes similar to those seen in viral latency. They induce a closed chromatin structure within the HIV-1 genome associated with methylation of H3K9 and H3K27, and recruitment of HDACs, resulting in nucleosome rearrangement and chromatin compaction and loss of RNA polymerase II. The RNA binding protein, Argonaute 1, complexed with the antisense strand of the siRNA is critical to this process, conferring sequence specificity and allowing preferential translocation to the nucleus of infected cells.

Effective delivery of these molecules is perhaps the greatest challenge facing the clinical development of this approach. Recently, it has been demonstrated that these complexes can be delivered as short hairpin RNAs after lentiviral transduction of CD4 T cells, and have been demonstrated that these complexes can be delivered as short hairpin RNAs after lentiviral transduction of CD4 T cells, and suppress virus even in in vitro models of uncontrolled HIV infection. Currently it is envisaged that, with other gene therapies of this type, these si/shRNAs would be administered as cell therapy to patients who have sustained viral suppression on ART. Once the cells have engrafted, ART would be ceased and the shRNAs produced from these cells would maintain viral latency, enforcing prolonged viral latency and perhaps a functional cure.

However, the optimisation of this process is not trivial. Currently, like all cell therapies, this involves harvesting CD4+ T cells and/or CD34+ stem cells from virally suppressed patients by leukapheresis, transduction of these cells with the gene therapy constructs in a purpose built laboratory, followed by reinfusion of these cells into patients. While this is challenging, the process is even more complicated. These cells need help to engraft effectively. Currently, the only available approach to this requires conditioning of the bone marrow with doses of chemotherapeutic drugs. This is a real challenge, even though the doses given are substantially reduced. Novel means of enhancing engraftment or alternative delivery vehicles such as nanoparticles or liposomes may simplify this process and are being explored. Therefore, although this approach is encouraging, like all the current strategies aimed at stabilising
the reservoir, there is much work to do and several substantial hurdles to clear before it is proven and practical therapy.

References

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Biography

Anthony Kelleher is a clinician scientist whose interests include the dissection of the immunovirology of HIV-1 infection to inform the development of vaccines and novel therapies.