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RNAi based gene therapy for HIV-1, from bench to bedside

Human immunodeficiency virus (HIV) can be successfully controlled with the current antiviral drugs. However, drug side effects and the development of viral drug resistance may cause problems and argue for the development of new HIV therapies. RNA interference (RNAi), discovered around a decade ago, provides the opportunity to develop a new antiviral therapy. RNAi is the mechanism in which small interfering RNAs (siRNAs) target RNA molecules with exquisite sequence-specificity, with subsequent cleavage or translational repression of the RNA. We use this mechanism to target the HIV RNA genome and viral mRNAs in infected cells. For this we use short hairpin RNAs (shRNAs), which are intracellularly processed into siRNAs. By treating the hematopoietic stem cells with shRNAs, all derived progenitor cells will be protected against HIV. It is expected that this protection by means of gene therapy will prevent the onset of AIDS in an HIV infected individual.

In this thesis we describe how HIV can escape from a single shRNA treatment directed against highly conserved viral sequences. Because we target highly conserved regions, the possible escape routes are restricted compared with shRNAs that are directed against non-conserved regions. Nevertheless, the virus can still escape by selecting a single point mutation in the target region. Viral drug resistance also occurs when a single antiretroviral drug is applied in a patient. Therefore, the current standard treatment consists of combinatorial antiretroviral therapy (cART). Our results underline the sequence-specificity of the RNAi-mechanism and the need for a combinatorial shRNA approach. To define optimal shRNA combinations we tested individual shRNAs for their potency to inhibit the virus using various highly sensitive assays. Several highly potent shRNAs were identified that yield viral inhibition for more than one hundred days. We have evaluated several methods for the expression of multiple siRNAs in a cell, such as the use of a single transcript that is processed into several individual siRNAs. We tested the safety and efficacy of the shRNA treatment in hematopoietic stem cells in a humanized immune system mouse model. The
lentiviral transduction and shRNA expression did not affect the ability of human CD34-positive hematopoietic stem cells to undergo hematopoiesis, but a temporal reduction in the number of shRNA expressing cells was observed in the thymus and the peripheral lymphoid organs. This research has contributed to the formulation of an optimal RNAi gene therapy for HIV. As such we have moved a step forward in the clinical application of an RNAi based gene therapy.