Silencing of HIV-1 co-factors

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Summary

The human immunodeficiency virus type 1 (HIV-1) is known for causing the disease “acquired immune deficiency syndrome” or AIDS. At the end of 2009 around 33 million people were infected worldwide and in the same year some 2.5 million people became newly infected. The disease can nowadays successfully be treated with a combination drug therapy, but this therapy comes with drawbacks; patients have to take drugs daily for the rest of their lives, the drugs can have severe side-effects, the medication is relatively expensive and they cannot cure the disease. Due to the high mutation rate of HIV-1 resistant virus variants emerged, rendering some drugs ineffective. With no vaccine in sight, new therapies need to be developed to combat HIV-1 infection.

In this thesis we developed RNA interference (RNAi) as an antiviral method. RNAi is a sequence-specific cellular mechanism that is induced by double-stranded RNA and that leads to gene silencing. RNAi has been already used successfully against HIV-1 RNA targets. T cell lines were transduced with lentiviral vectors encoding shRNAs against HIV-1 RNA and virus replication was inhibited in these cells. However, when a single HIV-1 sequence was viral targeted, resistance was observed, often by a point mutation in the targeted sequence. One of the solutions to prevent viral escape is to target the cellular proteins that HIV-1 requires to complete its replication cycle. Since HIV-1 encoded only 15 proteins, it relies on many cellular proteins, all representing possible therapeutic targets.

The best example is the co-receptor CCR5. It is known that a minority of the human population has a deletion in the CCR5-gene and thus do not produce an active CCR5 protein. These individuals are completely healthy, but virtually immune to infection by CCR5-tropic HIV-1 strains. There may be many more cellular co-factors that are indispensable for the virus, while their RNAi-mediated knockdown is harmless for the cell.

In this thesis we describe the use of RNAi against 30 different cellular co-factors (Chapter 3). T cells were treated with lentiviral vectors that encode a shRNA for co-factors silencing. First the cells were analyzed for negative effects of the transduction or shRNA expression on cell proliferation. To do so, the doubling time was measured and in 11 of 30 cases the knockdown was moderately to severely toxic for the cells; cells exhibited an increased doubling time or died. The remaining cells were infected with HIV-1 and the effects on HIV-1 replication were analyzed by measuring the production of HIV-1 in the culture supernatant. In some cases we could inhibit virus replication up to 2 months, indicating that RNAi against cellular cofactors can be used to strongly delay HIV-1 replication.

Since RNAi against a cellular co-factor can be potentially toxic, a sensitive method is necessary to score even small effects on cell growth. Available methods, such as counting cells, are labor-intensive and unreliable. We therefore developed a new and sensitive method that is based on competition in a culture between transduced and untransduced cells (Chapter 4). When cells are transduced with a lentiviral vector encoding a shRNA, a selection marker gets integrated as well. In most cases this is the GFP (green fluorescent protein) marker. When cells are transduced, the culture contains both transduced GFP-positive cells and untransduced GFP-negative cells. If transduced cells grow slower than normal cells (due to toxicity of the shRNA), this will result in a gradual decrease of the \( \text{GFP}^+/\text{GFP}^- \) ratio. We show that this method, the competitive cell growth (CCG) assay is more sensitive than existing methods and it was used in all further chapters.
Addendum

One of the top shRNAs in Chapter 3 targets a protein that is involved in the autophagy pathway. Autophagy is a cellular process that helps the cell to survive in cases of nutrient starvation. Many viruses rely on this pathway for their replication and although it already has been shown that autophagy plays a role in HIV-1 replication, the details remain elusive. In Chapter 5 we tested twelve autophagy proteins and found several of those to be important for HIV-1 replication. To improve the potency of virus inhibition, cells were transduced with lentiviral vectors encoding shRNAs against two autophagy factors.

It became clear in Chapter 6 that cellular proteins can play complex roles in HIV-1 replication. Earlier, a proteomics study was performed to analyze the up- and downregulation of protein expression in HIV-1 infected cells. Many of the hits from this study were further analyzed by assessing the effect of RNA-mediated knockdown of these proteins on HIV-1 replication. The focus of this chapter was on the cellular protein Cyclophilin B. The proteomics screen revealed that this protein is upregulated during HIV-1 infection, and knockdown enhances HIV-1 replication, suggesting that Cyclophilin B has an antiviral function. Interestingly, a closely related family member, Cyclophilin A, has an opposite function; HIV-1 requires this protein for replication. Both cyclophilins bind to the same part of the HIV-1 capsid protein. We propose that HIV-1 binds Cyclophilin A to prevent the antiviral activity of Cyclophilin B.

In Chapter 7 we analyzed the function of the cellular DDX3 protein in HIV-1 replication. DDX3 was already known to be involved in HIV-1 replication, as it helps in the nuclear export of HIV-1 RNAs. We show that DDX3 also influences HIV-1 gene expression by activating the viral Long Terminal Repeat promoter in a Tat-dependent manner. This makes DDX3 an attractive therapeutic target as it has multiple supportive roles in HIV-1 replication.

In Chapter 8 we describe different RNAi studies that have been described in literature to determine which cellular co-factors are used by a variety of viruses. For example, for influenza virus six studies have been done to determine the required host proteins. All studies report many candidate co-factors, but the overlap between these studies is surprisingly small. This shows that it is necessary to validate the initial results. Interestingly, viruses frequently present in the same patient, such as HIV-1 and hepatitis C virus, may require the same cellular co-factors. It may thus be possible to treat two viruses with a single drug. Caution is however warranted, e.g. for the treatment of both HIV-1 and tuberculosis, as it has been suggested to treat tuberculosis by induction of the autophagy pathway, whereas the opposite action seems needed to treat HIV-1 infection (Chapter 5). Cellular co-factors as novel antiviral therapeutic targets remain a viable option, but one has to be vigilant for these potential complications.