CONFERENCES

C2 | CONTROL OF MORBILLIVIRUS REPLICATION BY RNAi

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RNA interference (RNAi) is the process of mRNA degradation that is induced by double-stranded RNA in a sequence-specific manner. RNAi has a potential of developing into an effective and specific antiviral therapy.
Sixty two siRNA sequences targeting nucleocapsid (N) genes of peste des petits ruminants (PPR), rinderpest and measles viruses were designed, chemically synthesized and tested for efficacy *in vitro*. Three sequences were found to be most effective resulting in reduction of viral titer to about 1000 to 10000 times and also a decrease of about 20 to 150 times the number of copies of viral genome. There was also an inhibition of up to 90% of the nucleoprotein expression. In addition, these 62 siRNA duplexes were examined concerning the features associated to functionality. Our results suggest that the intra-molecular secondary structure of the targeted mRNA contributes to siRNA efficiency. It was confirmed that the presence of at least the sequence motifs U13, A or U19, as well as the absence of G13, cooperate to increase siRNA knockdown rates. It is also observed that G11 is linked with siRNA efficacy.
To test the efficacy for controlling PPRV infection in the infected animal, the siRNA (NPPRV1) found most active *in vitro* was selected. Various cationic lipid based formulations were tested in different mass ratios with siRNA in the presence of various percentages of goat serum and transfection times on Vero cell culture. In addition, a recombinant human adenovirus type 5 expressing shRNA NPPRV1 was constructed and tested on cell culture using various multiplicity of infection (MOIs). Transfection on cell cultures with lipoplexes could inhibit cytopathic effect of PPRV up to 45% in the presence of 60% goat serum. Adenovirus shRNA NPRV1 transduction at MOI of 80 could reduce PPRV titer by up to 2 logs.
For the *in vivo* experiment, thirty goats were divided in 3 groups of 10 animals. Group-1 and group-2 were injected intravenously with 0.5 X 10^{10} TCID50 of adenovirus shRNA NPPRV1 and an irrelevant adenovirus shRNA, respectively. They were challenged 48 hours later. Group-3 was injected with 3 doses of lipoplexes (12 mg siRNA/dose/day) and challenged 24 hours after first dose administration. Animals were examined daily for clinical signs and each sign was given a score. Blood and serum samples were collected for determining viremia (viral load) and serological studies. For diarrhea and temperature, both treatment groups had higher scores as compared to control group. While for nasal and lachrymal discharges, groups 1 and 3 on one hand and for signs of stomatitis, group 2 on the other hand, had lower scores than the controls. However, the difference was not statistically significant. The analyses for viremia and antibody level against PPRV and adenovirus are in process.
Currently, we are working on *in vivo* delivery strategies to improve the addressing of siRNAs to the target cells.

C3 | MOLECULAR BASIS OF PATHOGENESIS OF THE HERPESVIRUSES AND DEVELOPMENT OF HSV AS A GENE THERAPY VECTOR

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The herpesviruse family contains a number of ubiquitous human pathogens with the potential to cause severe disease. The herpesviruses establish life-long latent infections within specific cell types within the body that provide a reservoir for reactivation and maintenance of the infectious cycle. Herpes simplex virus type 1 (HSV-1) establishes a latent infection primarily within sensory neurons of the peripheral nervous system. While much is known about the regulation of the productive transcriptional program during a lytic infection, only recently have details regarding the mechanism of suppressing and regulating lytic gene expression in neurons during latency emerged. HSV-1 DNA is not methylated during latency, but there is evidence that specific histone modifications may play a central role in mediating lytic gene suppression. Recently we have demonstrated that the latent genome is organized into transcriptional domains that are defined by specific histone modifications that are either transcriptionally permissive (H3 H4dimethyl), or transcriptionally repressive (H3K9 dimethyl and H3K27 trimethyl). The fact that the histone modification H3K27dimethyl is associated with transcriptionally repression of the lytic genes suggests that the polycomb family of chromatin proteins plays a key role in silencing lytic genes during the establishment of latency. Moreover, we have recently shown that the cellular insulator protein CTCF binds to the HSV-1 genome during latency and acts to establish insulators that separate the transcriptional domains. This suggests that cellular proteins may act to suppress and regulate HSV-1 lytic genes in an organized and regulatable manner similar to how our genes are regulated on chromosomes. We have recently exploited these concepts to improve the development of HSV-1 vectors for gene therapy. Understanding the mechanism of regulating HSV-1 gene