CONTROL OF MORBILLIVIRUS REPLICATION BY RNAi

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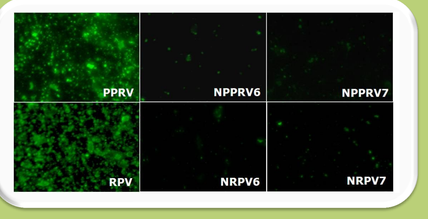
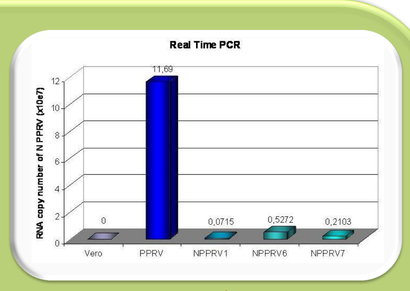
INTRODUCTION

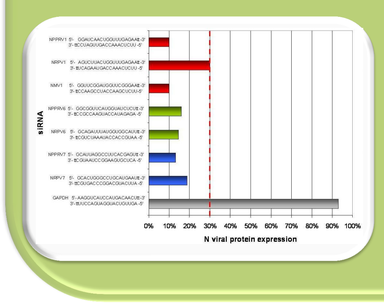
Peste des petits ruminants (PPR) and rinderpest (RP) are very important viral diseases of domestic and wild ruminants due to their highly contagious behaviour and their high rates of morbidity and mortality. Both viruses have an economic relevance in African and Asian countries and can infect large and small ruminant species. PPRV and RPV are members of the *Morbillivirus* genus in the *Paramyxoviridae* family. Although preventive vaccines are available against these diseases, efficient therapeutics for virus control under emergency situations are desirable. Inhibition of morbillivirus replication can be achieved by post-transcriptional silencing of the nucleoprotein (N) gene by RNA interference (RNAi). Sixty two siRNA sequences targeting three genes of PPRV, RPV and measles virus (another morbillivirus affecting humans) were designed, chemically synthesized and tested for efficacy *in vitro*. Three sequences directed against nucleoprotein (N) gene, were found to be most effective, resulting in about 1000 to 10000 fold reduction in viral titles and also decreasing the number of copies of viral genome by about 20 to 150 times. There was also an inhibition of viral nucleoprotein expression by up to 90 %. All results were consistent, suggesting that siRNA molecules could be developed as therapeutic agents for treatment of PPRV and RPV infections, if siRNAs can be efficiently delivered *in vivo*. For that, different viral and non-viral vectors are being tested as well as their stability to delivering molecules in presence of serum. For this purpose, a recombinant human adenovirus type 5 and a baculovirus both expressing the short hairpin RNA (shRNA) NPPRV1 were constructed and tested on cell culture using various multiplicities of infection (MOIs). A decrease of 2 logs in virus title was obtained using the adenovirus-NPPRV1. The baculovirus-NPPRV1 was capable to decrease around 70% of N protein expression *in vitro*. A peptidic agent used in cell transfection was also tested for *in vitro* siRNA NPPRV1 delivery in cell cultures and it was able to induce more than 90% of inhibition in viral N protein expression. This study also describes the investigation of the ability of PPRV to escape the treatment with the effective siRNAs, a major problem of all antiviral therapies. Indeed, after fourteen selections under siRNA NPPRV1 pressure a escape mutant was generated and after only four consecutive transfections with the siRNA NPPRV7 another escape mutant was obtained.

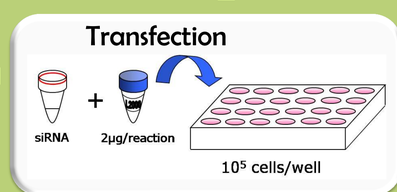
MATERIALS, METHODS AND RESULTS

Screening of siRNA sequences

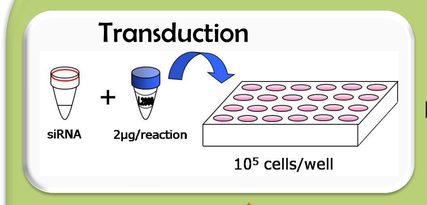


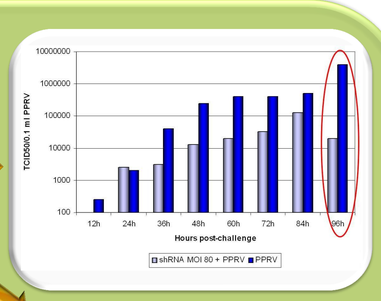


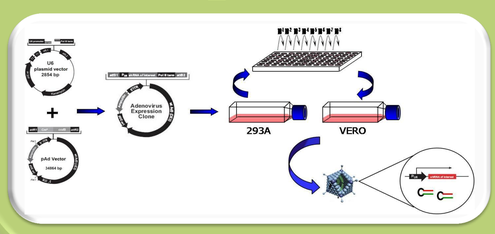




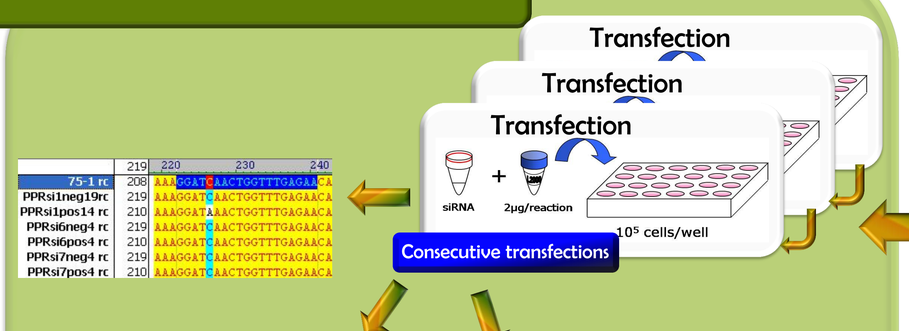
Adenovirus shRNA vector



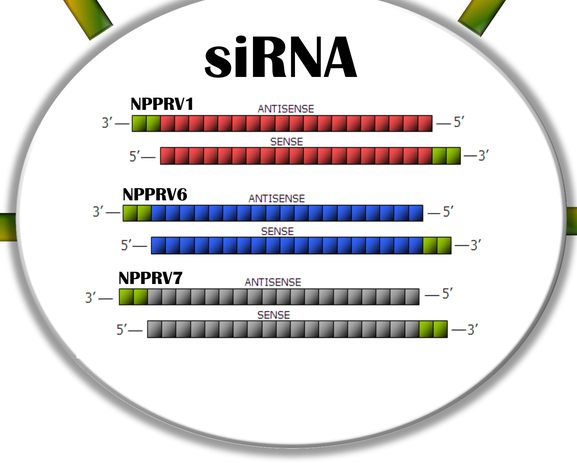




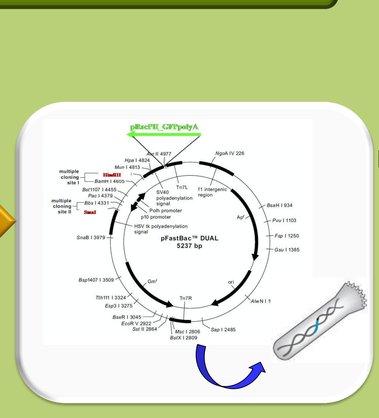
Research of escape resistant mutants

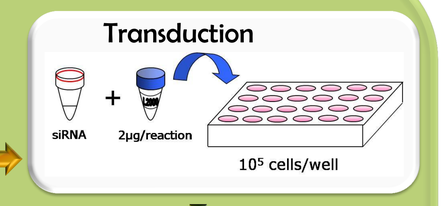


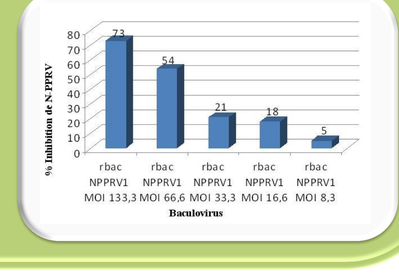
siRNA



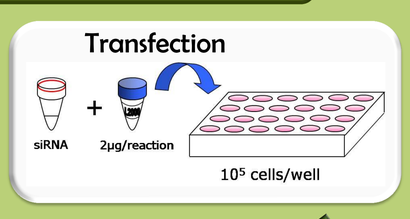
Baculovirus shRNA vector

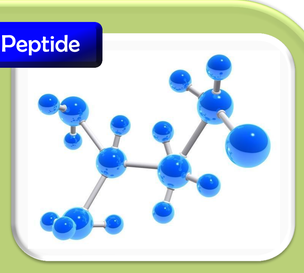


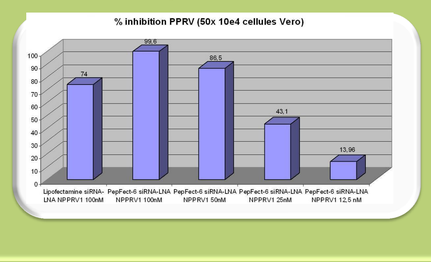




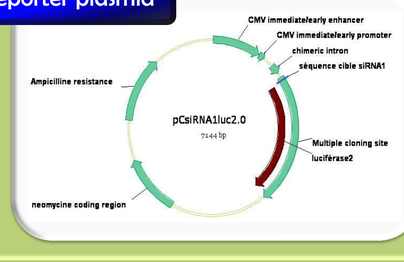
Non-viral vector: peptide



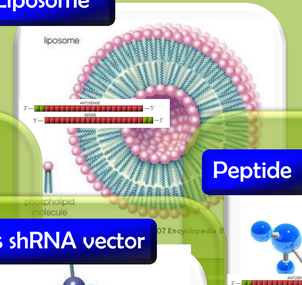




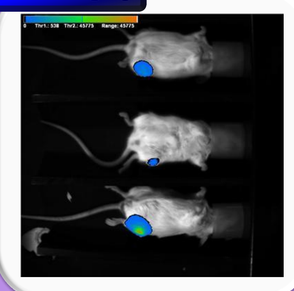
Reporter plasmid



Liposome



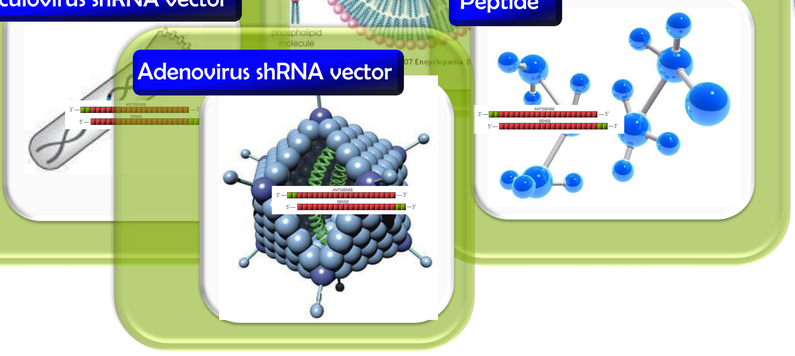
In vivo imaging



Baculovirus shRNA vector

Peptide

Adenovirus shRNA vector



PERSPECTIVES

The viral vectors developed in this work as well as other non-viral vehicles (a peptide and a lipoplex) will be tested as delivery systems of siRNANPPRV1 in mice. The activity of the siRNA delivered by these different formulations will be assessed on a reporter gene (currently under construction) and will be then measured by a bioimaging system in live animals. A model of systemic infection of mice by PPRV that could be used in bioimaging is also under investigation.

REFERENCES

Amarzguioui M.; Prydz H.; (2004). Biochem. Biophys. Res. Commun. 316:1050-1058.
Barik S. (2004). Virus Res. 102:27-35.
Reed L.J.; Muench H.A. (1938). Am. J. Trop. Med. Hyg. 27:493-497.
Servan de Almeida R.S.; Keita D.; Libeau G.;Albina E. (2007). J. Gen. Virol. 88:2307-2311.
Keita D.; Servan de Almeida R.S.; Libeau G.; Albina E. (2008). Antiviral Res. 80:158-167.

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