INTRODUCTION

Peste des petits ruminants virus (PPRV) is a morbillivirus responsible for a disease that affects sheep, goats, and some small wild ruminants species.

CIRAD previously identified three small interfering RNA that target conserved regions of the essential gene encoding the viral nucleoprotein and prevent at least 90% of PPRV replication in vitro. These molecules are currently under in vivo evaluation. However, there is an important risk of emergence of virus escape mutants since a single mutation can abrogate the RNA interference (RNAi).

OBJECTIVE

In this study, we investigated the ability of PPRV to escape the inhibition conferred by single or multiple siRNAs after several consecutive passages in vitro at suboptimal doses.

MATERIAL & METHODS

Vero cells were plated with different final concentrations of each siRNAs (100; 33.3; 11.1 and 3.7nM) complexed with Lipofectamine 2000 (Invitrogen). Twenty-four hours later, cells were infected with ten-fold dilutions of the virus collected in the previous transfection and the virus titration were realized. Four days later, the siRNA silencing effect was evaluated by scoring the reduction of CPE and the virus were collected, for to use in the next transfections and the characterization of the mutations by sequencing of the N gene and for the quantification of the mutant and wild virus populations by real-time PCR.

RESULTS

Except with the combination of the three different siRNAs, the virus systematically escaped RNAi after 3 to 20 consecutive passages (Table 1).

Mutations were characterized by single or multiple punctual nucleotide mutations (synonymous or not).

A deletion of a stretch of six nucleotides was also observed. While still complying with the so-called ‘rule of six’ in the morbillivirus genome for optimized replication, this deletion was shifted in the open reading frame (ORF). However, the shift only resulted in the loss of 2 amino-acids, the rest of the protein remaining unchanged.

REFERENCES


CONCLUSIONS

Several mutations were generated in the targets of our siRNA, allowing the virus to escape RNA interference. Sequence deletion often occurs when a non coding or non essential gene is targeted. However, in this work we were able to find for the first time an escape mutant with a deletion in an essential and highly conserved viral gene resulting in the production of a shorter protein.

This study provides new insights on the genomic plasticity of morbilliviruses that should be considered in antiviral strategies.

ACKNOWLEDGEMENT

This research was supported by Marie Curie International Fellowship. EPZONE, CIRAD and the Languedoc-Roussillon Region.