CONTROL OF AFRICAN SWINE FEVER VIRUS BY siRNA

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African Swine Fever (ASF) is a highly contagious, viral disease of pigs caused by an Asfivirus (Fig.1). It can cause up to 100% of mortality in domestic pigs and European wild boars, although varying degrees of virulence have been shown. In contrast, the infection is unapparent in African wild suids (warthogs, bush pigs, giant forest hogs). There is no treatment or vaccine to control this severe disease. To address this issue, we are currently working on the development of an attenuated vaccine and new generation of biological antivirals. For the generation of attenuated ASFV vaccine strains, we envisage to delete the 10 Kb region located 23638-33336 in the genome (Fig. 2), which includes nine genes that are thought to determine the virulence of ASFV. Of these 9 genes, 4 (A104R, A118R, A151R and A240L) have unknown function in the virus replication cycle. To establish the importance of these genes, we have used the RNA interference approach. RNA interference is a natural biological process initially described in plants [1]. It is a natural cell mechanism by which small interfering RNAs (siRNAs) of about 20 nucleotides operate to down regulate the expression of a gene by targeting and cleaving its mRNA (post-transcriptional gene silencing).

MATERIAL AND METHODS

Fig. 1: African Swine Fever Virus (ASFV): Asfivirus

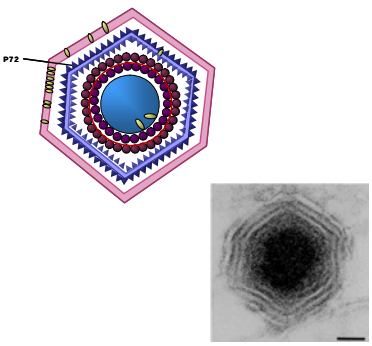
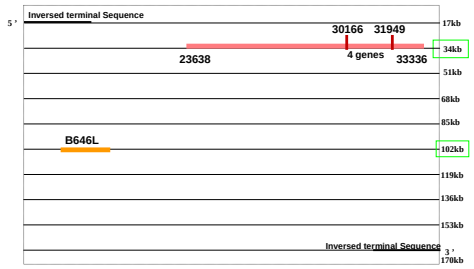


Fig. 2: Position of the 5 interesting genes in the ASFV genome



siRNA design

The four genes of interest (A104R, A118R, A151R and A240L), position 30166-31949 (Fig. 2) and also B646L (VP72) gene (as a control for essential gene), from a Spanish strain isolated in 1971 were cloned, sequenced and the sequence results were compared with the reference sequence available in GenBank (accession number ASU18466). The sequences were sent to Ambion-Applied Biosystems for siRNA design. Seventeen siRNAs were received, 3 were direct against each of the 4 genes of interest and 5 were direct against B646L mRNA.

siRNA-transfection

Vero cells were transfected with different doses of each of these siRNA using Qiagen HiPerfect reagent and 24 hours after, cells were infected with Ba71v virus. Three days later, siRNAs silencing effect were evaluated by measuring Ba71v Cytopathic Effect (CPE) and by virus titration.

CPE quantification

The siRNA effects were evaluated by direct microscopic observation of the cells and then after blue trypan staining. A score of CPE severity was attributed and being – for no CPE to +++ (> 70% of CPE).

Virus titration

To evaluate the virus progeny replication, ten-fold serial dilutions of the cell supernatant were inoculated onto Vero cells. The titre in $TCID_{50}$/ml was established according to Reed and Muench (1938).

Fig. 3: siRNA functionality measured by microscopic observation of CPE (A) and virus titration (A, B).

3.A

siRNA	VP72_1	VP72_2	VP72_3	VP72_4	VP72_5	A104R_1	A104R_2	A104R_3	A118R_1	A118R_2	A118R_3	A151R_1	A151R_2	A151R_3	A240L_1	A240L_2	A240L_3	Ba71v
CPE score	+++	+++	++	++	+++	+++	+++	+	+++	+++	++	+	+	+	+++	++	+	+++
Virus Titre (10^x $TCID_{50}$/ml)	5.9	6.1	4.5	4.7	5.1	5.5	5.1	4.3	5.7	5.5	5.5	3.9	2.9	3.5	5.1	4.3	4.3	5.9

RESULTS

siRNA functionality

Cytopathic effect was decreased by the three siRNA targeting the gene A151R, the siRNA A104R-3 and A240L-3 (Fig. 3.A). Accordingly, the virus titres were reduced by 1 to 3 $\log_{10}$ depending on the type and concentration of siRNA (Fig. 3.B). However, the effect of siRNA targeting the essential gene VP72 was surprisingly low. siRNA directed against A118R gene showed no or a very weak effect on the CPE and Virus Titres (Fig. 3).

The most efficient sequences were siRNA-A151R-2 and A151-3 with a clear CPE reduction (Fig. 4.A))and up to 2.4-3 log reduction of the virus titre (Fig. 4.B).

3.B

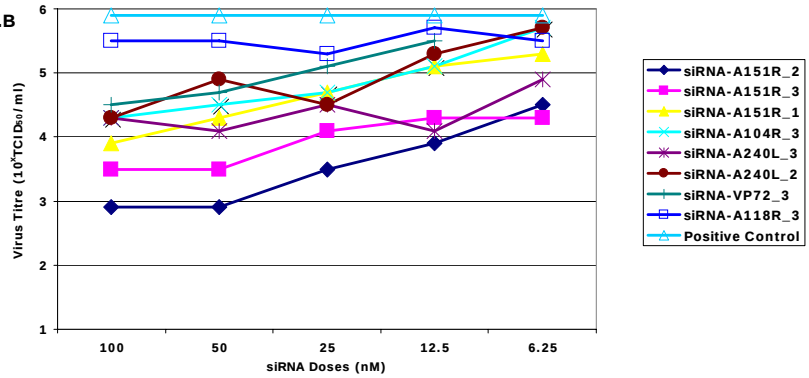
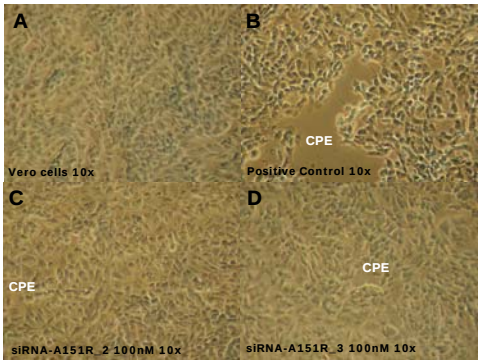
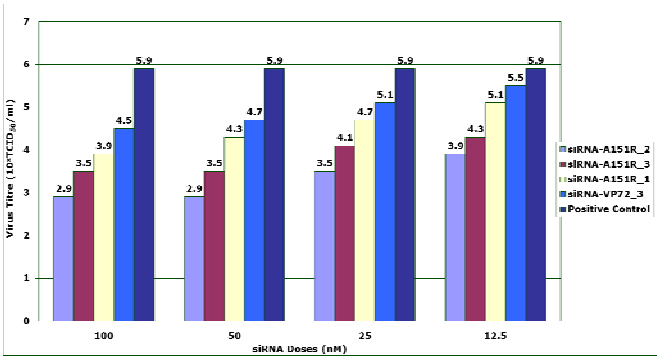


Fig. 4: siRNAs A151R silencing effect measured by microscopic observation (A) and virus titration (B).

4.A



4.B



DISCUSSION AND CONCLUSIONS

- In this study, we have evidenced the essential role of the A151R gene in Ba71v replication. Three siRNA were active against this gene with clear effect on virus replication. Therefore, an extra-copy of this gene will have to be inserted in the ASFv genome before attenuation by deleting the 10 kb which contain the original gene.
- The possibility to use these active sequences as potential antivirals in vivo will be also explored since in the absence of a vaccine, such strategy could become a useful alternative control strategy.
- The essential role of the three other genes of interest is not clearly established: some siRNA may have slight effect on CPE and virus titres, but further confirmation is needed. Thus, the effect of these siRNAs on the different gene transcripts will be soon assessed by real-time PCR.
- Finally, the lack of siRNA effect, in particular against the essential B646L (VP72) gene, may probably result from the incorrect design of the sequences. Further siRNA design is ongoing.

ACKNOWLEDGEMENT

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REFERENCES

1. Fire, A., Xu, S.Q., Montgomery, M.K., Kostas., S. A., Driver, S.E., Mello, C.C., 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391, 806-811.

2. Reed LJ, Muench, HA. A. (1938). Am. J. Trop. Med. Hyg., 27, 493-497.

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