Chemogenetic ON and OFF switches for RNA virus replication

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Summary

Therapeutic application of RNA viruses as oncolytic agents or gene vectors requires a tight control of virus activity if toxicity is a concern. Here we present a novel regulator switch for RNA viruses using a conditional protease approach, in which the function of at least one viral protein essential for transcription and replication is linked to autocatalytic, exogenous human immunodeficiency virus (HIV) protease activity. Virus activity can be en- or disabled by various HIV protease inhibitors. Incorporating the HIV protease dimer in the genome of vesicular stomatitis virus (VSV) into the open reading frame of either the P- or L-protein resulted in an ON switch. Here, virus activity depends on co-application of protease inhibitor in a dose-dependent manner. Conversely, an N-terminal VSV polymerase tag with the HIV protease dimer constitutes an OFF switch, as application of protease inhibitor stops virus activity. This technology could be applicable to any potentially therapeutic RNA virus.

MET AIMS

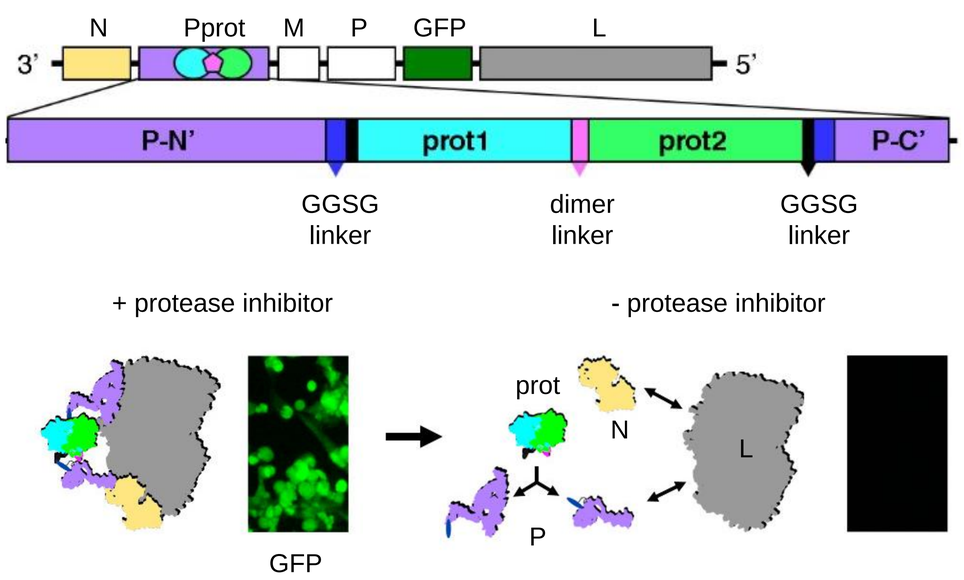
- Generate controllable viruses with clinically approved regulator compounds.

FUTURE AIMS

- Generate regulatable virus containing potentially toxic therapeutic transgenes.
- Show abrogation of transgene production in-vitro and toxicity in-vivo.

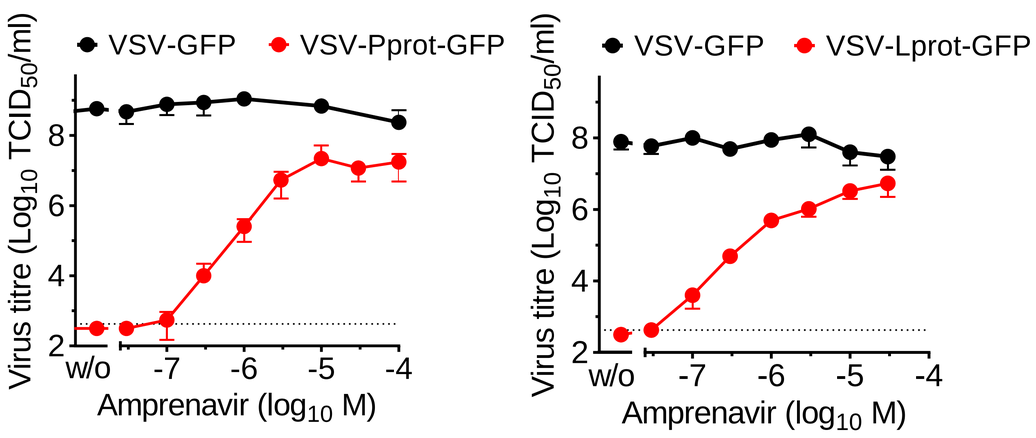
The Prot-ON principle

The autocatalytic HIV protease dimer inserted into proteins of the oncolytic and vaccine vector VSV leads to the cleavage of thus altered proteins. The addition of one of several clinically approved HIV protease inhibitors restores the proteins' integrity und virus function.

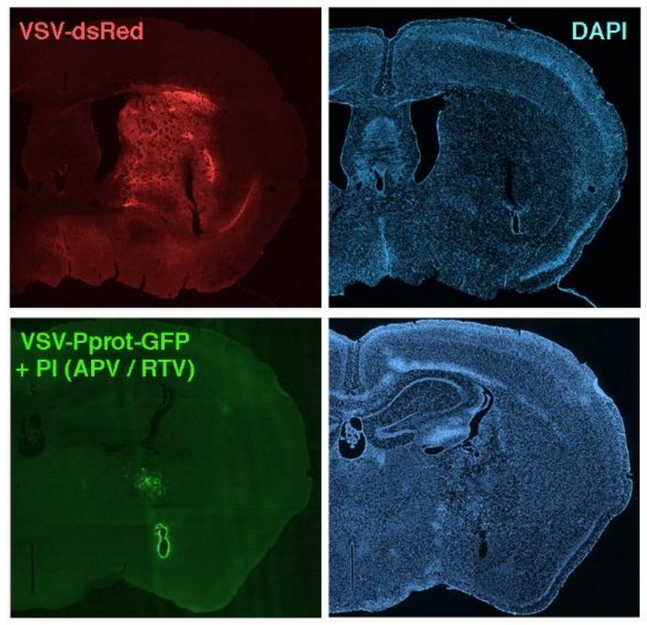


Results

Prot-ON viruses rely on the presence of protease inhibitors in an dose-depending manner. The virus titre as determined by tissue culture infection dose 50 ($TCID_{50}$) increases with the supplied amount of amprenavir, one of the first HIV protease inhibitors.

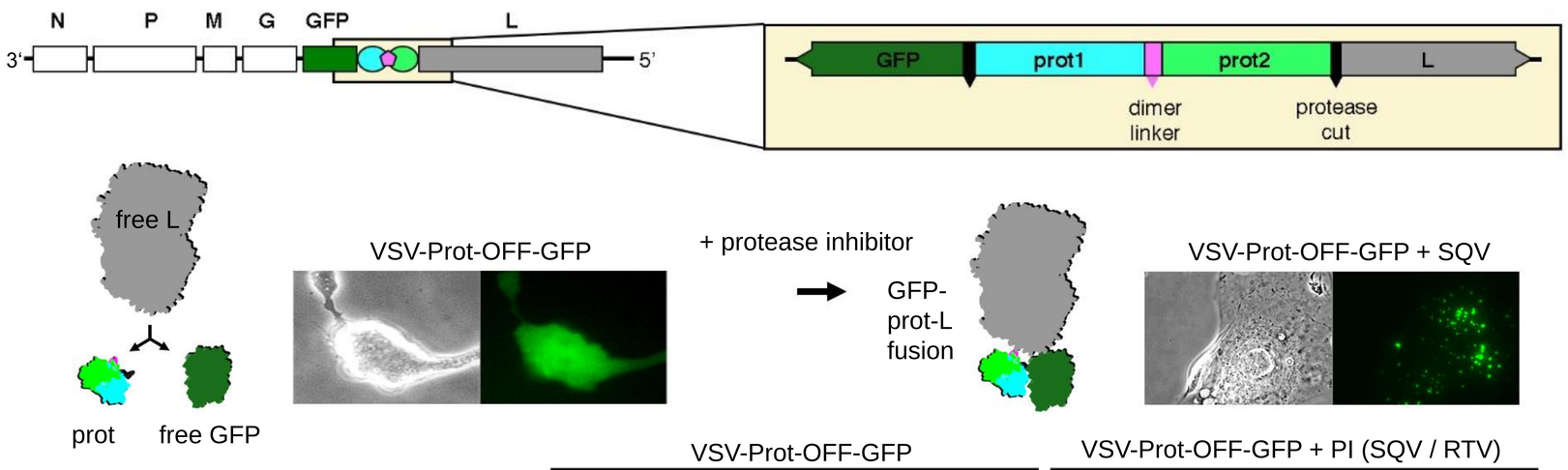


VSV is known for its pronounced neurotoxicity. We therefore investigated the Prot-ON viruses replication in the central nervous system. Only a minimal fraction of HIV protease inhibitors (PIs) such as amprenavir (APV) and ritonavir (RTV) reach the brain compared to their serum level. When we injected virus intracranially, GFP expression by Prot-ON virus was therefore confined around the needle tract. Contrary, dsRed-labeled wild-type virus spread quickly throughout the injected hemisphere, causing severe neurotoxicity in affected mice.



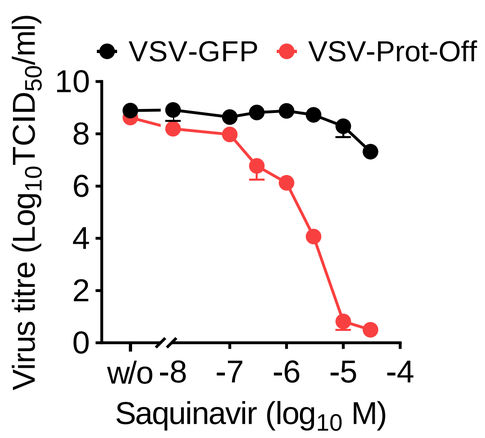
The Prot-OFF principle

The autocatalytic HIV protease dimer attached to one of the viral proteins, the large protein (L), blocks its polymerase function. Only in the absence of PIs the protease is released from the viral polymerase, the virus replicates and expresses GFP. Adding PI saquinavir (SQV) and virus at very high multiplicity of infection visualizes the trapped VSV replication machinery that locates to typical foci within the cytoplasm.



Results

Opposite to Prot-ON viruses, the Prot-OFF virus titre correlates inversely with the amount of a given protease inhibitor, e.g. saquinavir.



Tumors infected with VSV-Prot-OFF-GFP viruses were lysed by oncolytic action. Tumor cell nuclei stained with DAPI dissolve at sites of infection. Consequently, cavities of lysed tumor cells are filled with virus, visualized here with antibodies against the viral nucleoprotein (Anti-VSV-N). By intraperitoneal injection of protease inhibitors (PI) into tumor-bearing mice, virus spread was stalled. Thus, viral particles were mainly visualized at the site of injection.