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CRISPR/Cas9 as a tool for the study of the interactions between viruses and host

CRISPR/Cas9 is a prokaryotic molecular immunity system that exploits short RNAs to degrade complementary DNA sequences of invading bacteriophages. This system has been deeply characterized and engineered to be exploited for genome editing in eukaryotic cells.
CRISPR/Cas9 introduces specific double strand breaks in DNA molecules, activating two possible cellular repair responses. In the absence of a donor template, non-homologous end joining repair is activated, leading to insertion/deletion mutations that inactivate the target gene. In the presence of a homologous donor template, the damage is repaired triggering the homologous recombination pathway that inserts the donor sequence in the target locus. The CRISPR/Cas9 system has been applied, among others, to study and counteract viral infections.
CRISPR/Cas9 has been exploited to target the EBNA1 gene, reducing EBV latency1. Similar results were obtained on HBV2 and on HSV-1, by targeting the immediate-early genes, abrogating HSV-1 infectivity3,4. To avoid concerns associated with the use of porcine organs for transplantations, CRISPR/Cas9 has been applied to remove endogenous retroviruses from porcine cells5. It has been used to inactivate HPV-16/18, responsible of cervical carcinoma, by targeting the E6 and E7 genes6,7. The E7 gene was inactivated in HPV-6/11, the main causes of genital warts, within transformed keratinocytes8.
Several efforts have been employed to contrast HIV-1 infection. The CRISPR/Cas9 system has been developed to target the TAR sequence, conserved among different virus subtypes9,10, the HIV-1 regulatory Rev gene11 or the entire HIV-1 genome12. To avoid the generation of HIV-1 escape mutants13 a different strategy, targeting the CCR5 co-receptor, was employed14.
Our research group is studying the interactions between HIV-1 and cell host proteins. To define the role of MHC-I molecules in modulating HIV-1 infectivity, we developed β2microglobulin knock out cell lines. HIV-1 virions produced in β 2microglobulin negatives cells were found to be less infectious15. In addition, we showed that the HIV-1 Nef protein is stabilized by the human peroxisomal thioesterase 8 (ACOT8)16. To investigate the role of this interaction we developed ACOT8 negative cells to be used either for virus production and for viral infection.
CRISPR/Cas9 is thus a powerful system to study the interactions between viruses and host, as well as a promising therapeutic tool to fight viral infections.