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CRISPR RNA regulates apoCas9 mediated viral memorization in meningococci

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Background

Most prokaryotes rely on CRISPR-Cas for adaptive immunity against parasitic DNA. A functional CRISPR-Cas9 system exists in ~40% of meningococcal isolates and can restrict DNA natural transformation. Yet, the interplay between Neisseria CRISPR and phages is poorly understood. A handful of putative Neisseria filamentous (Nf) prophages have been identified, including the most noticeable meningococcal disease associated phage MDAΦ that is linked to hypervirulence and increased host colonization.

Aim/Methods

Using MDAΦ and the CRISPR+ strain Nme8013 as model, we aimed to investigate if, and how, CRISPR-Cas9 modulate the spread of Nf phages. We employ molecular genetics, high-throughput sequencing, and informatic approaches.

Results

MDAΦ lysogenizes the wildtype strain of Nme8013 either in an exclusively episomal state or as prophages integrated at dRS3 sites in the host genome. Engineered CRISPR-Cas9 that encodes a pre-existing MDAΦ-targeting spacer can abolish MDAΦ infection. This result demonstrated the role of CRISPR-Cas9 in restricting the spread of Nf phages in Meningococcus. Importantly, during successful MDAΦ lysogenization, CRISPR array can expand to have more spacers. These new memories are preferentially derived from across MDAΦ genome as opposed to the host genome. We showed that this memory repertoire expansion requires the cas1-cas2 integrase and cas9 effector genes but is independent of cas9's nuclease activity. Strikingly, mutant strains lacking the CRISPR RNA or tracrRNA, two essential RNA co-factors for Cas9-catalyzed DNA cleavage, exhibited greatly elevated spacer acquisition efficiencies. Through extensive genetic and NGS analyses, we demonstrated that this phenotype is due to apoCas9 (i.e., in RNA-free state) being a hyper-stimulator of viral memorization. Our findings also revealed a novel repressor role of CRISPR RNA and tracrRNA, to rein in apoCas9's hyper-activity in spacer acquisition. Such repression is important to mitigate auto-immunity risks caused by genomically derived self-targeting CRISPR spacers. Lastly, we examined if CRISPR can cure MDAΦ from a lysogen population. Using an inducible Cas9 system that allows us to activate Cas9 after MDAΦ infection is established, we showed that CRISPR-Cas9 can effectively cure MDAΦ episomes without killing the hosts.

Conclusions

CRISPR-Cas9 plays a vital role in modulating filamentous phage content and pathogenicity of Neisseria, by memorizing and blocking phage infections.