Harnessing the Prokaryotic CRISPR/Cas Foreign DNA Defense System for Antiviral Therapy in Canine Fibroblast Tumor Cells

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CRISPR/Cas is a prokaryotic defense system that produces complexes that target themselves to and degrade specific foreign nucleic acids, such as virus DNA. The CRISPR/Cas bacteria Streptococcus pyogenes was stimulated with the DNA of a eukaryotic virus (in this case, canine parvovirus [CPV]), and then the resulting CRISPR complexes produced were isolated and introduced into eukaryotic cells also infected with CPV. The hypothesis was that the complexes would be able to degrade the viral DNA present inside the cells, thereby providing a measure of antiviral resistance. However, host cell nucleases and proteases would eventually degrade the complexes, so resistance against the virus would not be permanent. To accomplish the experiment, CPV DNA was isolated, conjugated with cationic liposomes, and introduced into a S. pyogenes culture with their cell walls removed. After intervals of 1, 3, 6, and 9 hours from when liposomes were taken up, samples were taken out and cryolysed. The lysates were subjected to coimmunoprecipitation with Dynabeads and the cas9 (part of the CRISPR complex) antibody, and the resulting immunoprecipitates were then entrapped into liposomes. The liposome solution was introduced into CPV-infected canine fibroblast cells, and the cytopathic effects on the cells were compared to three different controls. Bacteria cell-wall removal and liposome formation were successful and verified by TEM imaging, while the immunoprecipitates were analyzed with a Nanodrop machine. The CPV-infected cells that were treated with the CRISPR complexes recovered significantly in viability compared to the non-treated infected cells, which displayed a consistent rate of cell loss.