CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

Vivek Kamarshi

Project Number

S1513

Project Title

Cellular Pathways for Increasing Fusion and Decreasing Replication of Varicella Zoster Virus

Abstract

Objectives

Varicella Zoster Virus (VZV) causes chicken pox and shingles. Cells infected with VZV undergo cell-to-cell membrane fusion mediated by viral fusion proteins; fusion is important to VZV pathology.
In a cell-based screening assay, the drug tacrolimus increased fusion by 300%--previously identified as being dependent on the drug complexing with a cellular protein. This drug-protein complex has documented effect of inhibiting the calcium-activated phosphatase calcineurin (CaN). My aims: 1) understand CaN s role in virus-modelling cell-cell fusion; 2) extend knowledge to live viral infections.

Methods

To quantify fusion, a cell-based model assay was used. To measure viral replication, skin-cell monolayers were inoculated with live virus, then evaluated for infection size/shape. CaN activity's relationship to fusion was investigated using drugs. Cyclosporin inhibits CaN similarly to tacrolimus; drugs affecting CaN's upstream activation were also used, including Ionomycin (increase intracellular calcium concentration and thus hyper-activates CaN) and Nicardipine (decreases calcium, resulting in hypo-activation).

Results

When cyclosporin was added to assay experiments it increased cell-cell fusion. Nicardipine was found to increase fusion, whereas ionomycin decreased fusion. This is consistent with the theory that level of Calcineurin activity is negatively correlated with cell-cell fusion in the assay.
To describe the effects of calcineurin activity, cells were stained for NFAT, a CaN substrate protein. When ionomycin was added to cells, NFAT translocated from the cytoplasm to the nucleus (indicating dephosphorylation); addition of a CaN-inhibitor blocked this effect. This confirms CaN's phosphatase role.
In vitro live-viral infections in the presence of CaN-inhibitors showed proportionally large amounts of infected-cell detachment and plaques with smaller overall size, as compared to controls. Thus, drugs which inhibit Calcineurin activity change live virus-induced cell fusion and reduce viral spread in cell monolayers.
Finally, an alternate fusion protein, when used in the fusion assay, saw inconsistent effects on fusion upon CaN inhibition. This suggests calcineurin inhibitors hyper-fusogenic effects are VZV-specific.

Conclusions

This is the first ever approach for reducing a live virus' spread by changing cell fusion. My findings that fusion is virus-specific and that CaN behaves as expected confirm that this approach to therapies is medically relevant. CaN-inhibitors make poor antivirals because they have immunosuppressive properties in vivo; however, a downstream step of this pathway might create a new target for an anti-VZV drug.

Summary Statement

I created a potential new approach to treating Varicella Zoster Virus, identifying a cellular protein that interfaces with the virus' "cell-cell fusion" effect and demonstrating that drugs which inhibit that protein can slow viral spread.

Help Received

I would like to thank Drs. Stefan Oliver, Marvin Sommer, and Momei Zhou (Stanford Peds Infectious Diseases, Arvin Lab), who allowed me to use lab facilities and lent mentorship on experimental design and data analysis. Thanks also to Ms. Renee Fallon and Mr. Pooya Hajjarian for presentation advice.