CALIFORNIA SCIENCE & ENGINEERING FAIR
2018 PROJECT SUMMARY

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38574

Project Title
Tacrolimus-Induced Changes in Fusogenicity of Varicella Zoster Virus

Abstract

Objectives/Goals
Varicella Zoster Virus (VZV) causes chicken pox and shingles. Cells infected with VZV undergo cell-to-cell membrane fusion mediated by viral fusion proteins. The rate of cell-cell fusion has been found important to VZV pathology; increased fusion hurts virus propagation.
In a screening assay, the drug tacrolimus increased fusion by 300%. My project: Through which cellular pathways does tacrolimus increase cell-cell fusion?

Methods/Materials
To quantify fusion, a cell-based model of virus infection: viral fusion proteins are transiently expressed in reporter melanoma cells, which glow upon fusing.
Tacrolimus and other "macrolide" drugs bind FKBP proteins in the cell. This binding inhibits FKBPs' natural activity, but FKBP-drug complex also has cellular interactions. I used a shRNA system to knock down FKBP1A in melanoma cells. I generated three different shRNA cell lines, with 95%, 82%, and 88% decreases in cellular FKBP1A levels.

Results
I found that in the absence of VZV fusion proteins, no macrolide caused cell fusion. Tacrolimus has a dose-dependent effect on fusion: 10uM drug causes fusion increase ($P<0.0001$), 0.1uM drug has an insignificant effect. Thus, tacrolimus' effect on fusion occurs through clinically relevant pathways. Out of the macrolides, drugs tacrolimus and pimecrolimus elevate fusion ($P<0.0001$ at 2.5 and 5uM drug), while everolimus and sirolimus decrease it ($P=0.0229$, 0.0581 at 1.25uM). Decrease of cellular FKBP1A correlated with less elevated cell-cell fusion from pimecrolimus. The drug cyclosporin increased fusion ($P=0.001$ at 2.5uM).

Conclusions/Discussion
Increased fusion due to tacrolimus-related drugs was shown to depend on presence of FKBP. Therefore, the downstream interactions of the FKBP-macrolide complex result in the observed change in fusion.
Note: tacrolimus or pimecrolimus complexes inhibit Calcineurin phosphatase [CaN] and everolimus or sirolimus complexes inhibit mTOR kinase. Thus, the findings that only tacrolimus and pimecrolimus increased fusion, and that (alternate-mechanism) CaN inhibitor cyclosporin also increased fusion, suggest that inhibition of CaN increases cell-cell fusion.
As in vivo immunosuppressants, CaN-inhibitors are not ideal anti-VZV drugs. However, if the fusion-related downstream step of this pathway (potentially one of the proteins which CaN dephosphorylates) can be identified, this would create the target for a new fusion-increasing, anti-VZV drug.

Summary Statement
I validated that tacrolimus mediates an increase in varicella zoster virus-caused cell-cell fusion, and traced the effect to inhibition of Calcineurin by a tacrolimus-protein complex; this creates a new target for anti-VZV drug research.

Help Received
I would like to thank Drs. Stefan Oliver and Marvin Sommer (Stanford Pediatrics Infectious Diseases, Arvin Lab), who allowed me to use their lab facilities and gave mentorship on experimental design and data analysis throughout the project. Thanks to Ms Renee Fallon for presentation advice.