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Medical Sciences Comprehensive Exam 2019-04-22

Summary of Research Proposal

Herpes simplex virus (HSV) is a double stranded DNA virus that can cause mucosal lesions in humans during its lytic phase and that persists in its latent phase as a life-long infection inside neural ganglia. In most cases, it is not life-threatening, and painful lesions are the furthest extent of its burden. When complications do occur, however, they can be very serious, and include things like infection of a newborn causing birth defects, obstructive urethral swelling, a higher risk of additional sexually transmitted infection through lesions, and meningitis. The massive burden of these rare complications exists due to the sheer volume of people infected – over half of the global population.

Lipid bilayer capsules called extracellular vesicles (EV) are cells' natural intercellular carriers of signalling and functional cargo including proteins, miRNAs, mRNAs, and DNAs. EV have gathered considerable attention in recent years due in part to their aptitude as intercellular vehicles of cargo and in part to the fact that they do not elicit an immune response. In 2011, the group Alvarez-Erviti *et al*. (10) discovered that by expressing the rabies viral glycoprotein (RVG) as a fusion protein with lysosome-associated membrane glycoprotein 2 (LAMP2B) on an EV membrane surface, rabies neurotropism could be conferred to those EV.

Hypothesis: I hypothesize that extracellular vesicles expressing the LAMP2B-RVG fusion protein and containing anti-HSV compounds will target neurons and combat latent HSV infection *in vitro* and *in vivo*.

Methods: The latent phase of HSV is difficult to contend with due to its self-induced transcriptional and translational repression. I will first identify the optimal cargo of EV to target HSV in its heterochromatic state by testing 5 different cargo types loaded into EV via electroporation on latently infected human cells *in vitro*. I will next determine the accuracy with which LAMP2B-RVG-EV mimic the natural neurotropism of HSV-1 following entry to the body at both the oral and vaginal mucosa. This will be done using a luciferase bioluminescence assay on BALB/c mice to test different potential entry points for LAMP2B-RVG-EV. Lastly, I will determine the ability of LAMP2B-RVG-EV with a nuclease targeted to the UL19 and UL30 genes of HSV-1 to cripple the ability of HSV to resume the lytic phase of its lifecycle following latency. This will be measured as counts of lesions 4 weeks after the initial treatment on a hairless guinea pig and measuring viral RNA concentrations in guinea pig spinal fluid compared to a control receiving no treatment.

Impact: This research will compare the immunologically inert LAMP2B-RVG-EV with its rival adeno-associated virus 8 (AAV8) counterpart to see which cargo delivery system is best for the treatment of HSV. We will also learn the appropriateness of LAMP2B-RVG-EV to shadow the movement of HSV inside neurons *in vivo*. This data will bring us one step closer to developing a safe, immune-inert delivery system for drug treatment to the neurons where HSV hides. The virus that plagues greater than half the world's population has been considered a peripheral problem for too long.