Public Abstract

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Title:CELL-TO-CELL INFECTION, CELL-CELL FUSION AND PRODUCTION OF EBOLAVIRUS: MECHANISMS OF ACTION AND CELLULAR MODULATORS

Ebolavirus (EBOV), a member of the Filoviridae family, is a deadly non-segment negative-sense RNA virus that causes hemorrhagic fever among human and other species; the fatality rate can be up to 80%. In 2014, a re-emergent and serious outbreak of EBOV occurred in West Africa, which had claimed more than 11,000 human deaths. Although antibody cocktails and life supports were available, many people, including scientists and clinicians, still have had suffered and died of EBOV infection. Hence, a better understanding of EBOV biology, in particular its interaction with the host, is of critical importance. The purpose of my Ph.D. thesis project is to gain a better understanding of EBOV glycoprotein (GP)-mediated fusion, entry, and cell-to-cell transmission, and VP40-mediated production, with particular focus on the interplay between EBOV proteins and host factors that modulate this process.

We first established a cell-to-cell transmission assay by co-culturing viral producer cells and target cells. We found that EBOV utilizes cell-cell contact for efficient spread between cells, the process of which is dependent on GP. The cell-to-cell transmission of EBOV is more efficient than the cell-free infection performed in parallel. By applying inhibitors known to block cell-to-cell transmission and neutralizing antibody, we found that cell-to-cell transmission mediated by EBOV GP is sensitive to these inhibitors but relatively resistant to KZ52. Interestingly, we found that, even without the expression of viral core (e.g. MLV-Gag protein or EBOV VP40), EBOV GP is sufficient to support transfer of Tet-off protein from donor cells to target cells. Consistent results are also obtained in the context of replication competent rVSV bearing GP as well as EBOV VP40-based VLPs.

In collaboration with Fredric Cohen’s lab at Rush University, we established a cell-cell fusion assay for EBOV and investigated the role of potential triggers involved in the viral fusion process. We found that low pH is not a direct trigger of GP-mediated fusion yet the activities of Cathepsin are required. We observed that binding of EBOV GP to the endosomal receptor NPC1 is critical for enlargement of fusion pore and fusion kinetics.

We performed a series of experiments to determine the role of an antiviral protein, Viperin, in the replication of EBOV. We found that Viperin efficiently inhibits the replication of replication-competent virus-like particles (trVLPs). Consistently, knockdown of endogenous Viperin increases the replication of EBOV trVLPs in human lung cancer A549 cells. Mechanistically, we found that Viperin specifically reduces the expression of EBOV VP40 by inducing autophagy-mediated lysosomal degradation. Consistent with this finding, autophagy induction by Rapamycin significantly inhibits the replication of EBOV trVLPs, in association with reduced viral production. We examined the effect of some non-human primate Viperin proteins, and found that the antiviral activity against EBOV is conserved in these species, suggesting that Viperin may have contributed to the evolution and transmission of EBOV in primates.

In summary, we have determined the mechanisms of action of EBOV GP-mediated cell-cell transmission and cell-cell fusion, and defined a novel role of Viperin in inhibiting EBOV replication and production. Our work will help better understand the EBOV biology, its interactions with the host, as well as the development of new therapeutics against EBOV diseases.