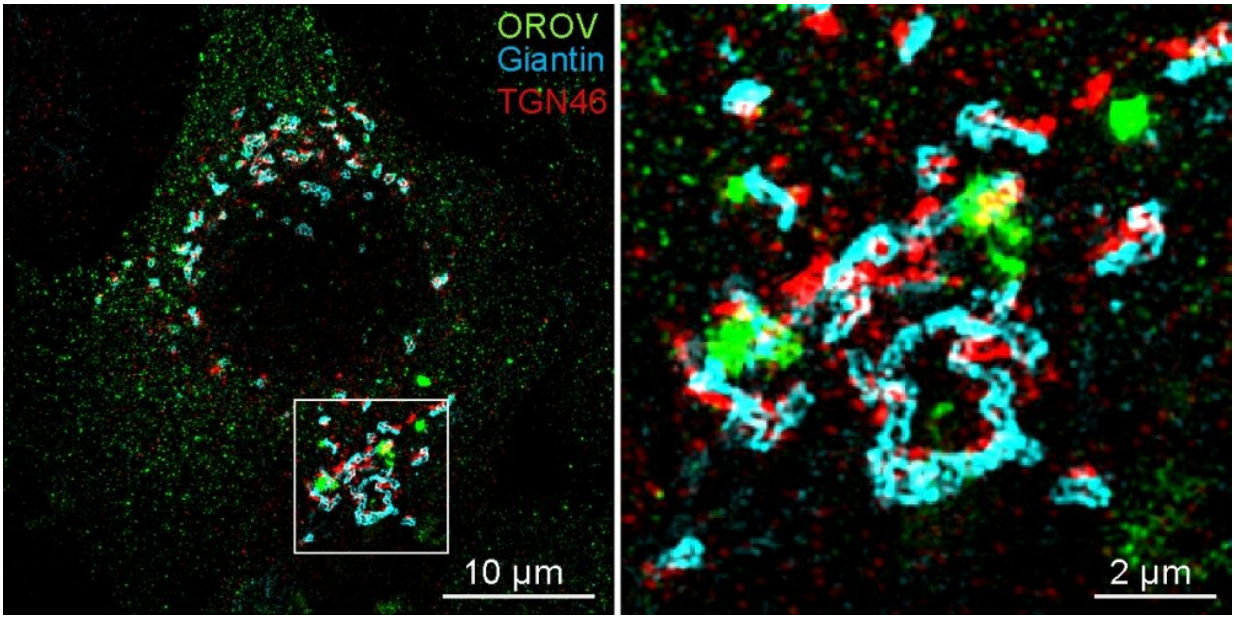
Study shows how Oropouche virus replicates in human cells

July 31 2018, by Karina Toledo



Results described in PLOS Pathogens point to potential targets worth exploring in effort to halt infection (HeLa cells stained to Oropouche virus [green], TGN46 and giantin proteins [red and blue], 18 hours after being infected / *PLOS Pathogens*

The strategy used by the Oropouche virus to replicate in human cells has been described for the first time by researchers at the University of São Paulo (USP) in Brazil and international collaborators in an article published in the journal *PLOS Pathogens*.

According to the study, shortly after invading the cell, the pathogen "hijacks" an organelle called the Golgi complex, which becomes a veritable virus factory. The virus does this by recruiting host cell protein complexes known as ESCRT (pronounced "escort"), which are capable of deforming the organelle's membrane and allowing the viral genome to penetrate it.

"This method of hijacking the Golgi complex via the use of ESCRT proteins had never been demonstrated for any other virus. It's a discovery that points to novel targets for exploration in the effort to prevent infection," said Natalia Barbosa, a Ph.D. researcher affiliated with USP's Ribeirão Preto Medical School (FMRP-USP) and the first author of the article.

The study was supported by the São Paulo Research Foundation—FAPESP and supervised by Luis Lamberti Pinto da Silva, a professor at FMRP-USP. Scientists at Tübingen University Hospital in Germany collaborated.

According to Silva, very little is known about the replication mechanisms of viruses in the family Peri bunyaviridae, to which Oropouche virus belongs.

"They're important pathogens from the public health standpoint," he said. "In Brazil, only Oropouche virus causes disease, but La Crosse encephalitis virus and Crimean Congo virus, which causes hemorrhagic fever, are endemic in other parts of the world. There are also members of the family that cause disease in cattle."

The symptoms of Oropouche virus infection are similar to those of dengue, consisting mainly of joint pain, headache, pain behind the eyes, and high fever. The difference is that in approximately half of all cases, a relapse of the disease occurs after the symptoms improve.

The virus is transmitted by Culicoides paraenses, a biting midge with urban habits. Outbreaks in villages and towns in the Brazilian Amazon are estimated to have caused a half-million cases, but Oropouche has also cropped up in other parts of the country and is considered an emerging virus by experts.

"The disease is certainly underreported, as it's often confused with other arboviruses," said the FAPESP scholarship supervisor. "It's rated as low severity, but the concern is that we don't yet know whether and how much the infection harms the nervous system in the long run."

In vitro experiments performed by the FMRP-USP group showed that the virus can infect neurons in mice and hamsters. The researchers are now trying to reproduce the experiments using human nerve cells. The principal investigator for this study is Eurico Arruda, a member of FMRP-USP's Virus Research Center and a coauthor of the article.

"Oropouche appears to be capable of infecting various types of cell. In other words, it succeeds in interacting with different receptors located on the surface of human cells. However, we don't yet know which receptors are used by any member of the family Peri bunyaviridae," Silva said.

To investigate Oropouche's replication mechanisms, the FMRP-USP group performed in vitro experiments with HeLa cells, the oldest and most widely used line in laboratories, derived from a human patient's cervical cancer cells.

"As soon as the cells are infected, the virus starts producing proteins that attract the host's ESCRT complexes to the external membrane of the Golgi complex. These ESCRT proteins then push on the organelle's membrane, rupture it and sweep into the Golgi complex, taking the viral genome with them. So the virus replicates inside the complex. What probably happens then is that some time later, the modified organelle

full of viruses merges with the plasma membrane and releases the pathogens into the extracellular medium," Silva said.

Other viruses are known to recruit the ESCRT machinery in order to replicate. For example, HIV, the pathogen that causes AIDS, uses ESCRT proteins to cross the plasma membrane that separates the intracellular and extracellular mediums. "However, this mechanism had never been described for invasion of the Golgi complex by viruses," Silva said.

The Golgi complex is a series of stacked membranes and vesicles whose main function is to process, store and distribute proteins produced in ribosomes. "We don't know exactly how this hijacking of the Golgi complex affects the host cell, but the HeLa cells die some 36 hours after being infected," Silva said.

In a previous study led by Arruda, the group showed that Oropouche produces a protein called NSs that induces apoptosis, a process of programmed death, in the host cell. "This protein isn't part of the virus's structure, and we don't know how killing the host cell by apoptosis benefits the pathogen, but it could be the result of a defense mechanism," Arruda said. "The NSs protein in isolation can cause apoptosis, and its use could be explored to kill tumor cells, for example."

Possible targets

In one of the experiments described in the *PLOS Pathogens* article, the researchers manipulated HeLa cells so that they no longer expressed Tsg101, an important ESCRT protein. To do this, they used RNA interference, a method of blocking gene expression by inserting short RNA sequences into cells.

"This intervention made HeLa cells more resistant to infection by

Oropouche. They took longer to die and had a much smaller viral load. There are experimental drugs that inhibit Tsg101, and we're now going to test them against Oropouche," Silva said. Because Tsg101 is a key protein in normal human cell function, he added, it may not be possible to use drugs that inhibit it or other ESCRT proteins to treat patients. The risk of adverse side effects would be considerable.

"However, there may be a molecule that inhibits interaction between the virus and human proteins without impeding the activity of Tsg101 in cells. This deserves to be studied," he said.

The FAPESP-funded research also plans to determine which proteins are produced by Oropouche to recruit the ESCRT complex. "They would also be potential targets worth exploring to halt the infection," Silva said.

More information: Natalia S. Barbosa et al, ESCRT machinery components are required for Orthobunyavirus particle production in Golgi compartments, *PLOS Pathogens* (2018). DOI: 10.1371/journal.ppat.1007047

Provided by FAPESP