Hunting for HCMV receptors a never ending story?

Laurent Perez
Institute for Research in Biomedicine, Università della Svizzera italiana, Via Vincenzo Vela 6. CH-6500 Bellinzona, Switzerland.
Correspondence: laurent.perez@irb.usi.ch

Human cytomegalovirus (HCMV) is a successful, widespread pathogen with an elevated sero-prevalence of 80% in humans worldwide[1]. HCMV primary infection of healthy individuals is usually asymptomatic, but establishes lifelong latency. However, infection *in utero* of the developing fetus is the leading viral cause of congenital birth defects. Furthermore, viral reactivation in the immunocompromised patient can lead to severe clinical symptoms, such as disseminated viral infection, blindness and potentially cancer. Virtually, all cell types can be infected and it is known that HCMV initiates infection by low affinity binding to cell surface heparan sulfate proteoglycans[2]. HCMV tethering to HSPGs is followed by a high affinity binding to specific cellular receptors, attachment is determined by two viral glycoproteins complex, the gHgLgO trimer and the gHgLpUL128/UL130/UL131A pentamer[3]. Numerous specific receptor candidates have been proposed over the last decades. However, none of these receptors have been found to be absolutely necessary for infection of the susceptible cell types[4].

Viral receptor identification is always a complicated task, pathogens often used multiple pathway to infect the host. Furthermore, the extremely broad tropism of HCMV and the usage of a laboratory adapted strain rendered its receptor identification more than challenging. HCMV laboratory strains are widely used since they propagate efficiently in fibroblasts and large virus titers can be obtained. Nonetheless, these strains contain genomic deletions, mutations and lack the pentameric glycoprotein complex required for broad tropism infection[5]. As a major consequence of this deficiency, laboratory strains that efficiently infect fibroblasts cannot infect epithelial cells. From this observation, we reasoned that fibroblasts might potentially express a receptor, that is absent in epithelial cells. To address this hypothesis, we compared gene expression profile of fibroblasts versus epithelial cells and investigated differences in signaling pathways activated upon viral exposure. We discovered from this set of experiments that PDGFRα was present and activated upon viral stimulation in fibroblasts only. To further validate this finding and confirm PDGFRα as one HCMV receptor, we had to generate several key reagents such as recombinant viral proteins, cell lines over-expressing or knock down for different tyrosine kinases receptors. Indeed, we demonstrated that the knock down of PDGFRα abolish fibroblasts infection by the HCMV laboratory strain, while PDGFRα expression in epithelial cells rendered them permissive to the HCMV laboratory strain.

To gain insight into the molecular mechanism of HCMV trimer/PDGFRα interaction, we initiated collaboration with Peter Kwong laboratory at the NIH who could visualize the viral trimeric gHgLgO complex and identify that gO is the key subunit for the interaction with PDGFRα.

Surprisingly, we also discovered that the trimer was required not only for fibroblasts infection but also for epithelial cells infection even if we showed that epithelial cells do not express PDGFRα. This finding indicates that the gHgLgO trimer possesses additional functions and might be necessary for viral infection of all cell types. Indeed, recent results proposed the trimer as an activator of the viral fusion[6].

We did identify one of the HCMV receptor, but is it really the end of the story? Of course not. Several important questions remain about HCMV entry. We demonstrated in our study that ErbBs receptors are functionally involved in HCMV infection and this seems to be dependent on the pentamer complex, although we could not yet find any evidence of a direct physical interaction between pentamer and ErbBs, therefore we cannot consider them as true HCMV receptors. Identification of a physical receptor for the pentamer (if it exists) will still require investigation. In addition, understanding how the trimer can trigger the gB fusion protein to perform viral fusion will also need additional investigation.

Answers to both questions will be of major interest to understand HCMV entry in the host.

Reference

1. Kenneson, A. & Cannon, M.J. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Rev Med Virol* **17**, 253-276 (2007).
2. Compton, T., Nowlin, D.M. & Cooper, N.R. Initiation of human cytomegalovirus infection requires initial interaction with cell surface heparan sulfate. *Virology* **193**, 834-841 (1993).
3. Ciferri, C*., et al.* Structural and biochemical studies of HCMV gH/gL/gO and Pentamer reveal mutually exclusive cell entry complexes. *Proc Natl Acad Sci U S A* **112**, 1767-1772 (2015).
4. Vanarsdall, A.L. & Johnson, D.C. Human cytomegalovirus entry into cells. *Current opinion in virology* **2**, 37-42 (2012).
5. Wang, D. & Shenk, T. Human cytomegalovirus UL131 open reading frame is required for epithelial cell tropism. *J Virol* **79**, 10330-10338 (2005).
6. Zhou, M., Lanchy, J.M. & Ryckman, B.J. Human Cytomegalovirus gH/gL/gO Promotes the Fusion Step of Entry into All Cell Types, whereas gH/gL/UL128-131 Broadens Virus Tropism through a Distinct Mechanism. *J Virol* **89**, 8999-9009 (2015).