Characterization of Enterovirus A71 entry factors in cell lines and Human Enteroids using CRISPR-Cas9 Knock-Out

LAYMAN'S SUMMARY:

Enterovirus A71 (EV-A71) is a non-enveloped, positive single-stranded RNA virus belonging to the *Picornaviridae* family, which is considered a major public health concern due to recent global outbreaks[1–3]. EV-A71 is one of the main causative agents of hand, foot and mouth diseases (HFMD)[4] and it can cause illness in multiple organs, such as the gastrointestinal and respiratory tract[5]. Most worrisome however, is EV-A71's ability to infect the central nervous system (CNS)[6]. It is considered to be the most neurotoxic enterovirus[7] due to potential life-threatening consequences in young children. Despite this, the entry mechanism EV-A71 utilizes to enter the host cell and initiate infection remains elusive. Thus, fully understanding the factors involved in viral entry in the primary replication site, the gastrointestinal tract, is a crucial step towards combating EV-A71 infection.

Multiple receptors have been shown to be involved in EV-A71 infection over the years, such as SCARB2[8] and HSPGs[9]. SCARB2, a lysosomal protein, is believed to play a role in uncoating the viral capsid[10], as well as act as an entry receptor[11]. However, due to SCARB2's low expression in the cell membrane[12], its role as an entry receptor is unlikely. Thus, other factors, such as HSPG2, might fulfil this role. Most studies on EV-A71 entry have been performed in cell lines, models which present multiple crucial limitations[13–15]. To overcome these limitations, enteroids, which are human 3D tissue cultures mimicking the function and structure of the intestine[16], are promising tools for the study of EV-A71 infection. Therefore, in this study we aimed to characterize the role of SCARB2 and HSPG2 in EV-A71 infection using more physiologically relevant models, enteroids, as well as cell lines. To do so, we utilized CRISPR-Cas9 technology, to knock-out SCARB2 and HSPG2 in these models, which allows for the loss of expression of these proteins.

Here, we showed SCARB2 plays a pivotal role in EV-A71 infection, since loss of SCARB2 expression significantly hindered EV-A71 infection in both cell lines and enteroids. Furthermore, SCARB2 loss did not affect the binding ability of EV-A71 to the cell membrane, nor did it hinder the internalization of viral particles inside the cell. Thus, our study presents SCARB2 as an essential factor for EV-A71 infection only post-internalization, disproving SCARB2's role as an entry receptor demonstrated in previous reports. Following these findings, we sought to assess another protein which was suggested to be involved in EV-A71 entry, HSPG2. Our findings showed HSPG2 loss did not impact EV-A71 binding to the cell membrane nor internalization into the cell, hence HSPG2 is not an entry receptor of EV-A71. Furthermore, EV-A71 infection was not hindered after loss of HSPG2 expression in neither cell lines nor enteroids. Thus, in our study HSPG2 did not play a role in any steps of EV-A71 infection.

Overall, we have demonstrated the crucial role of SCARB2 in EV-A71 infection and showed its role is limited to post-internalization stages. Furthermore, we showed EV-A71 infection is independent of HSPG2. These findings, shed a light on specific host proteins in the early stages of EV-A71 infection in physiologically relevant models. Finally, it stresses the need to further characterize EV-A71's entry pathway in order to fully understand EV-A71 viral pathogenesis.

References

1. Wang J, Hu Y, Zheng M. Enterovirus A71 antivirals: Past, present, and future. *Acta Pharmaceutica Sinica B*. 2022;12(4):1542-1566. doi:10.1016/j.apsb.2021.08.017
2. Autore G, Bernardi L, Perrone S, Esposito S. Update on Viral Infections Involving the Central Nervous System in Pediatric Patients. *Children (Basel)*. 2021;8(9):782. doi:10.3390/children8090782

3. de Ceano-Vivas M, García ML, Velázquez A, et al. Neurodevelopmental Outcomes of Infants Younger Than 90 Days Old Following Enterovirus and Parechovirus Infections of the Central Nervous System. *Frontiers in Pediatrics*. 2021;9. Accessed December 7, 2022. https://www.frontiersin.org/articles/10.3389/fped.2021.719119

4. Nayak G, Bhuyan SK, Bhuyan R, Sahu A, Kar D, Kuanar A. Global emergence of Enterovirus 71: a systematic review. *Beni Suef Univ J Basic Appl Sci*. 2022;11(1):78. doi:10.1186/s43088-022-00258-4

5. de Crom SCM, Rossen JWA, van Furth AM, Obihara CC. Enterovirus and parechovirus infection in children: a brief overview. *Eur J Pediatr*. 2016;175:1023-1029. doi:10.1007/s00431-016-2725-7

6. Lee KY. Enterovirus 71 infection and neurological complications. *Korean J Pediatr*. 2016;59(10):395-401. doi:10.3345/kjp.2016.59.10.395

7. Enterovirus 71. Accessed June 18, 2023. https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/enterovirus-71

8. Tang Q, Xu Z, Zhang F, et al. Identification of a novel binding inhibitor that blocks the interaction between hSCARB2 and VP1 of enterovirus 71. *Cell Insight*. 2022;1(2):100016. doi:10.1016/j.cellin.2022.100016

9. Tan CW, Poh CL, Sam IC, Chan YF. Enterovirus 71 Uses Cell Surface Heparan Sulfate Glycosaminoglycan as an Attachment Receptor. *Journal of Virology*. 2013;87(1):611-620. doi:10.1128/JVI.02226-12

10. Dang M, Wang X, Wang Q, et al. Molecular mechanism of SCARB2-mediated attachment and uncoating of EV71. *Protein Cell*. 2014;5(9):692-703. doi:10.1007/s13238-014-0087-3

11. Yamayoshi S, Ohka S, Fujii K, Koike S. Functional Comparison of SCARB2 and PSGL1 as Receptors for Enterovirus 71. *Journal of Virology*. 2013;87(6):3335-3347. doi:10.1128/JVI.02070-12

12. Kobayashi K, Koike S. Cellular receptors for enterovirus A71. *Journal of Biomedical Science*. 2020;27(1):23. doi:10.1186/s12929-020-0615-9

13. Hynds RE, Vladimirou E, Janes SM. The secret lives of cancer cell lines. *Dis Model Mech*. 2018;11(11):dmm037366. doi:10.1242/dmm.037366

14. Lawko N, Plaskasovitis C, Stokes C, et al. 3D Tissue Models as an Effective Tool for Studying Viruses and Vaccine Development. *Frontiers in Materials*. 2021;8. Accessed December 14, 2022. https://www.frontiersin.org/articles/10.3389/fmats.2021.631373

15. Mouriaux F, Zaniolo K, Bergeron MA, et al. Effects of Long-term Serial Passaging on the Characteristics and Properties of Cell Lines Derived From Uveal Melanoma Primary Tumors. *Investigative Ophthalmology & Visual Science*. 2016;57(13):5288-5301. doi:10.1167/iovs.16-19317

16. Sang Y, Miller LC, Nelli RK, Giménez-Lirola LG. Harness Organoid Models for Virological Studies in Animals: A Cross-Species Perspective. *Frontiers in Microbiology*. 2021;12. Accessed December 14, 2022. https://www.frontiersin.org/articles/10.3389/fmicb.2021.725074