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Commentary

Could an endo-lysosomal ion channel be the Achilles heel of SARS-CoV2?

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ABSTRACT

The ongoing SARS-CoV2 outbreak has developed into a global pandemic. Despite previous outbreaks of SARS-CoV and the related MERS-CoV in recent years, neither a vaccine nor any other medication for an effective treatment are currently available. Endo-lysosomal two-pore cation channels have now emerged as potential novel targets for SARS-CoV treatment.

The great hero of Greek mythology, Achilles, was described as extraordinarily strong and courageous, but he had one weak point - his heel. His mother Thetis had gripped him tightly by the foot while dipping him into the river Styx, whose waters were said to confer invulnerability. Achilles thus acquired invulnerability, with the exception of his heel. Achilles later on participated in the Trojan war in the course of which, famously, the Greeks offered Troy a magnificent wooden horse as present, hiding their soldiers within the monument, thus bypassing the invulnerable Trojan walls and eventually defeating the Trojans. Similarly, viruses can employ a 'Trojan horse-like' strategy to enter the host cell. They hijack different receptors and thus evade the barrier imposed by the plasma membrane [1]. In case of SARS-CoV and SARS-CoV2 this receptor is angiotensin-converting enzyme 2 (ACE2), while MERS-CoV recognizes dipeptidyl peptidase 4 (DPP4) as its receptor [2] (Fig. 1). Once inside the cell, the virus must evade the second line of defense, digestion in acidic lysosomes, by escaping the endo-lysosomal system. The onward trafficking of cargo such as viruses (i.e. from early endosomes and phagosomes to late endosomes and finally lysosomes) relies on the function and activity of several proteins including Rab proteins, SNAREs (SNA(P) R)ceptor) proteins) such as syntaxins and VAMPs which mediate vesicle fusion, and ion channels, in particular the two-pore cation channels TPC1 and TPC2. TPC1 and TPC2 reside in complexes with different Rab and SNARE proteins in endo-lysosomal vesicles; TPC1 preferentially in early endosomal compartments, TPC2 in late endosomal/lysosomal compartments [3–5] (Fig. 1). Genetic ablation of TPCs or TPC blockers have been previously shown to affect trafficking of both viruses and bacterial toxins through the endo-lysosomal system, reducing infectivity, including, e.g. cholera toxin, diphtheria toxin, *Pasteurella multocida* toxin, Anthrax toxin, Ebola virus, and MERS-CoV [5–7]. Ou et al. now show that inhibition of PIKfyve significantly reduces entry of MERS-CoV, SARS-CoV, and SARS-CoV2 S pseudovirions by endocytosis [8]. PIKfyve is an enzyme which phosphorylates PI3P to $PI(3,5)P_2$, a phosphoinositide highly abundant in endo-lysosomal membranes, regulating endo-lysosomal vesicle trafficking. $PI(3,5)P_2$ is a potent endogenous activator of TPCs and mucolipins (TRPML1, 2 and 3, alike TPCs serving as endo-lysosomal cation channels permeable for e.g. calcium and sodium). Ou et al. probed these downstream effectors of $PI(3,5)P_2$ by applying antagonists against these channels. Tetrandrine, previously described to block Ebola virus (EBOV) trafficking in a TPC-dependent manner [6], was found to block SARS-CoV2 pseudovirion entry. In contrast, an inhibitor of TRPML1 (albeit lacking information on chemical structure and IUPAC nomenclature) was found to be ineffective. These observations are in line with results by Gunaratne et al. [7], who previously demonstrated that pharmacological inhibition or knockdown of TPCs, but not TRPML1, impaired infectivity in a MERS-CoV spike pseudovirus particle translocation assay. From their data, Ou et al. concluded that inhibition of TPC2 would block SARS-CoV2 pseudovirion entry. Several points however remain unanswered. Tetrandrine blocks TPC1 and TPC2, and both are activated by $PI(3,5)P_2$. To corroborate their hypothesis that specifically TPC2 is involved in CoV entry, experiments genetically ablating TPC1 versus TPC2, or experiments using selective blockers for TPC1 versus TPC2, would be helpful. Along similar lines, it needs to be further defined whether blockage of early endosomal or late endosomal/lysosomal trafficking may be more relevant for SARS-CoV infectivity. Indeed, it has been demonstrated before that SARS-CoV, like EBOV, displays late cell entry kinetics and that transport to NPC1 + (Niemann-Pick C1 protein) late endo-lysosomes is a rate-defining step

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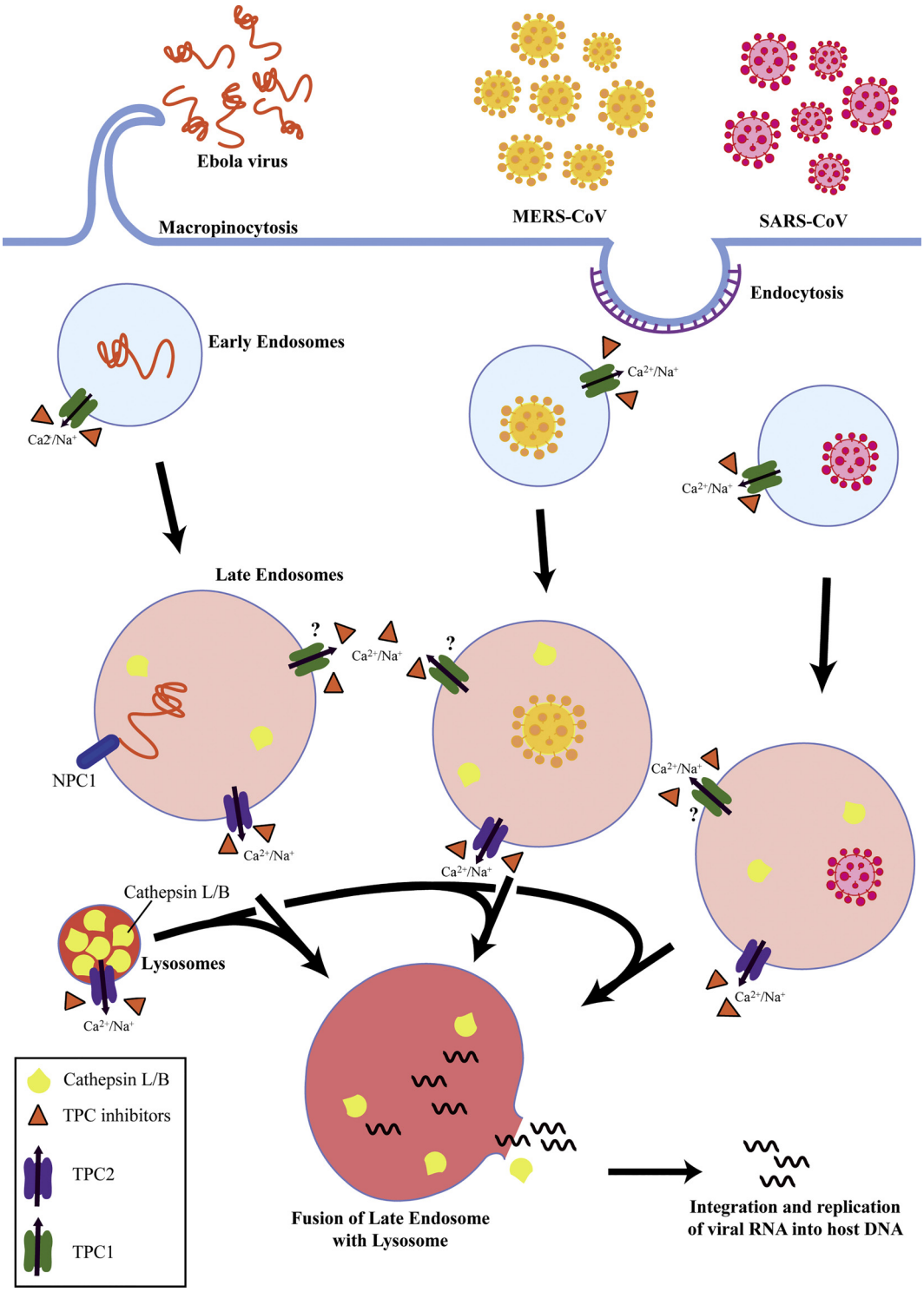

Fig. 1. Two-Pore Channels (TPCs) involved in EBOVs, MERS-CoV and SARS-CoV life cycle through the endo-lysosomal system. The two-pore channels (TPC1, TPC2) are required by viruses such as EBOV (Ebola virus), MERS-CoV and SARS-CoV, orchestrating the interplay of virus and endo-lysosomal milieus such as trafficking from early to late endosomes, fusion of late endosomes with lysosomes, and facilitating releasing of viral RNA into the cytoplasm. Unlike MERS-CoV and SARS-CoV being engulfed by cell membrane (clathrin-mediated endocytosis), EBOV enters the endo-lysosomal system after macropinocytosis. Early endosomes mature to late endosomes and fuse with lysosomes, delivering cathepsins and V-type H^+ ATPases. Viral glycoproteins (GPs) are cleaved by cathepsins in the acidic, cathepsin-rich environment, permitting their binding to endosomal membrane proteins, such as NPC intracellular cholesterol transporter 1 (NPC1) for EBOV. TPC antagonists affect trafficking between endo-lysosomal organelles with TPC2 inhibition affecting in particular the late endosome step of EBOV GP proteolysis, preventing virus entry into the cytoplasm, and retaining the virus within within late endosomes endosomes. Similar interactions can be envisaged for MERS-CoV and SARS-CoV, which also require cathepsin GP cleavage for cytosolic entry. Eventually, replication begins after release of viral mRNA into the host cytoplasm. Thereby, it appears that bisbenzylisoquinoline alkaloids such as Tetrandrine (Ebola/MERS-CoV) and Fangchinoline (MERS-CoV), blocking TPCs, allow manipulation of endo-lysosomal channel functions, impairing viral translocation and uptake into the cytoplasm.

[9]. As demonstrated for EBOV, both TPC1 and TPC2 are involved in virus trafficking and infectivity. The same may apply here. Finally, to fully exclude a role of TRPML1, the other target of $PI(3,5)P_2$, knockout or knockdown experiments are warranted. Furthermore, a variety of thoroughly characterized TRPML blockers have been described, such as ML-SI1 and ML-SI3, which would support the notion that SARS-CoV2 entry is TRPML-independent [10]. Unfortunately, the chemical nature of the blocker used by Ou et al., compound "130″ is not revealed, and endo-lysosomal patch-clamp experiments or other functional data to demonstrate that this compound indeed blocks TRPML1 are missing. Nevertheless, the data by Gunaratne et al. [7] and Ou et al. [8] suggest that TPCs may indeed be promising targets to interfere with CoV infectivity, and that it is well worth to further explore this in more detail in future studies.

Returning to Achilles and his single vulnerable spot, his heel: Paris, the son of king Priam and queen Hecuba of Troy, finally killed the seemingly invincible Achilles during the Trojan war with an arrow right into his heel. SARS-CoV2 may have several such heels, maybe one of these is the two-pore channels which, when targeted specifically, may help to efficiently combat the virus.

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References

[1] J. Staring, M. Raaben, T.R. Brummelkamp, Viral escape from endosomes and host detection at a glance, J. Cell. Sci. 131 (2018) 1–8, https://doi.org/10.1242/jcs.216259.

[2] W. Tai, L. He, X. Zhang, J. Pu, D. Voronin, S. Jiang, Y. Zhou, L. Du, Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine, Cell. Mol. Immunol. (2020), https://doi.org/10.1038/s41423-020-0400-4 https://www.nature.com/articles/s41423-020-0400-4.

[3] Y. Lin-Moshier, M.V. Keebler, R. Hooper, M.J. Boulware, X. Liu, D. Churamani, M.E. Abood, T.F. Walseth, E. Brailoiu, S. Patel, J.S. Marchant, The Two-pore channel (TPC) interactome unmasks isoform-specific roles for TPCs in endolysosomal morphology and cell pigmentation, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 13087–13092, https://doi.org/10.1073/pnas.1407004111.

[4] C. Grimm, L.M. Holdt, C.-C. Chen, S. Hassan, C. Muller, S. Jors, H. Cuny, S. Kissing, B. Schroder, E. Butz, B. Northoff, J. Castonguay, C.A. Luber, M. Moser, S. Spahn, R. Lullmann-Rauch, C. Fendel, N. Klugbauer, O. Griesbeck, A. Haas, M. Mann, F. Bracher, D. Teupser, P. Saftig, M. Biel, C. Wahl-Schott, High susceptibility to fatty liver disease in two-pore channel 2-deficient mice, Nat. Commun. 5 (2014) 4699–4712, https://doi.org/10.1038/ncomms5699.

[5] J. Castonguay, J.H.C. Orth, T. Müller, F. Sleman, C. Grimm, C. Wahl-Schott, M. Biel, R.T. Mallmann, W. Bildl, U. Schulte, N. Klugbauer, The two-pore channel TPC1 is required for efficient protein processing through early and recycling endosomes, Sci. Rep. 7 (2017) 1–15, https://doi.org/10.1038/s41598-017-10607-4.

[6] Y. Sakurai, A.A. Kolokoltsov, C.-C.C. Chen, M.W. Tidwell, W.E. Bauta, N. Klugbauer, C. Grimm, C. Wahl-Schott, M. Biel, R.A. Davey, Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment, Science 347 (2015) 995–998, https://doi.org/10.1126/science.1258758.

[7] G.S. Gunaratne, Y. Yang, F. Li, T.F. Walseth, J.S. Marchant, NAADP-dependent Ca2+ signaling regulates Middle East Respiratory Syndrome-Coronavirus pseudovirus translocation through the endolysosomal system, Cell Calcium 75 (2018) 30–41, https://doi.org/10.1016/j.ceca.2018.08.003.

[8] X. Ou, Y. Liu, X. Lei, P. Li, D. Mi, L. Ren, L. Guo, R. Guo, T. Chen, J. Hu, Z. Xiang, Z. Mu, X. Chen, J. Chen, K. Hu, Q. Jin, J. Wang, Z. Qian, Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV, Nat. Commun. (2020) 1620, https://doi.org/10.1038/s41467-020-15562-9.

[9] R.M. Mingo, J.A. Simmons, C.J. Shoemaker, E.A. Nelson, K.L. Schornberg, R.S. D'Souza, J.E. Casanova, J.M. White, Ebola virus and severe acute respiratory syndrome coronavirus display late cell entry kinetics: evidence that transport to NPC1+ endolysosomes is a rate-defining step, J. Virol. 89 (2015) 2931–2943, https://doi.org/10.1128/jvi.03398-14.

[10] W. Wang, Q. Gao, M. Yang, X. Zhang, L. Yu, M. Lawas, X. Li, M. Bryant-Genevier, N.T. Southall, J. Marugan, M. Ferrer, H. Xu, Up-regulation of lysosomal TRPML1 channels is essential for lysosomal adaptation to nutrient starvation, Proc. Natl. Acad. Sci. 112 (2015) E1373–E1381, https://doi.org/10.1073/pnas.1419669112.