Title:	Perturbation of vesicular trafficking by diphenylurea derivatives or by LIN7 gene silencing robustly attenuates influenza A virus host cell entry
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Abstract:	Influenza A virus (IAV) is a significant human respiratory pathogen that represents a serious threat to global health by causing recurrent epidemics and occasional pandemics. Protection against the virus can be achieved through seasonal immunization, but rapid evolution of the virus and the emergence of immune-escape variants pose major challenges in the long-term sustenance of vaccine-driven immunity. Although vaccination remains a cornerstone in prophylaxis, antivirals constitute a cardinal element in both chemoprophylaxis and treatment. Majority of the currently available anti-influenza drugs target the virus-encoded proteins, but the emergence of drug-resistant variants has threatened the canonical treatment regime, compelling identification of novel strategies to overcome antiviral resistance. In the fight against influenza, host-directed therapy is an emerging approach. In this approach, instead of targeting the virus, the host factors or pathways that are exploited by the virus are temporarily silenced to block infection. The advantage of host-directed agents over the canonical virus-targeting antivirals is their less likelihood of inducing resistance and their broader effectiveness against multiple viral strains. In the host-directed approach, the host cell entry of viruses represents a promising target for antiviral development. Blocking viruses at the entry step should inhibit infection early on, preventing viral replication and transmission. This study was directed to identify novel anti-influenza compounds with broad-spectrum potency and elucidate the function of a host protein that we identified as a critical mediator of IAV entry, which can serve as a potential antiviral target. In the first part, we conducted high-content small molecule screens against IAV infection, and identified five diphenylurea derivatives (DPUDs) that almost completely neutralized several IAV strains without inducing any significant cytotoxicity. Although DPUDs did not interfere with IAV binding to the cell surface, they robustly attenuated viral internalization and penetration at the late endosome. We found that DPUDs do not target the virus, but affect the host endosomal pathways and endosomal acidification, arresting the viruses at the entry step. The chemical properties of the compounds suggested that they are potential chloride transporters. Since intracellular chloride plays a critical role in regulating vesicular trafficking, we addressed whether the observed impairment of the endosomal pathways was due to the chloride-transport activity of the DPUDs. Using large unilamellar vesicles with chloride-sensitive dye and by expressing chloride-sensing fluorophores in cells, we demonstrated that DPUDs indeed transport chloride ions across the cell membrane, leading to intracellular chloride accumulation, which possibly leads to vesicular trafficking defects. VThe breadth of the inhibitory potential of the DPUDs was tested on other viruses including SARS-CoV-2. Remarkably, DPUDs almost completely blocked the cell entry of the wild type SARS-CoV-2 and its major variants of concern. Finally, we tested the DPUDs in mice challenged with IAV and mouse-adapted SARS-CoV-2 (MA 10). Treatment of the infected mice with the DPUDs led to remarkable body weight recovery, improved survival and significantly reduced lung viral load, highlighting their potential as broad-spectrum antivirals. In the second part, we elucidated the function of a host protein, LIN7B, in IAV infection. LIN7B was initially identified as a hit in an RNAi screen targeting the ‘druggable genome’. LIN7B was previously known to function in polarity determination in epithelial cells and coupling synaptic vesicle exocytosis to cell adhesion in neurons. We found that RNAi- mediated knockdown of LIN7B restricts IAV internalization, consequently leading to a block in infection. Although LIN7B was known as a cytosolic protein, we found by microscopy and biochemical analysis that it is also present on endosomes and it regulates endosomal trafficking of IAV. Interestingly, we also found that LIN7B deficiency leads to actin dysregulation, vesicular trafficking defects, and reduction in the surface distribution of EGFR, a major receptor for IAV. Through elucidation of the critical role of LIN7B in IAV infection, we illuminate the previously unknown functions of this protein in the regulation of cytoskeletal elements and vesicular trafficking.
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