Enhancing Endosomal Escape of siRNA Drugs by Proton Conductance via M2 Channel of Influenza A Virus

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RNA therapy has shown great potential for treating various genetic diseases, but the lysosomal degradation of intracellular RNA drugs due to low endosomal escape remains a significant challenge. To address this issue, we developed a novel virus-like delivery platform for siRNA drugs that incorporates the proton channel of the influenza A virus to enhance endosomal escape. We utilized M2 transmembrane (M2TM) domain as both a mediator for tethering RNA to the surface of the liposome and a conductor for absorbing protons into the liposome. We demonstrated that tethered RNA enhances the internalization of virus mimicking liposome (VML) into the cells via interaction with scavenger receptor A. Proton conductance through M2TM and M2siRNA complex embedded in lipid bilayer of liposome was confirmed by proton flux assay. Our in vitro assays for trafficking the endosomal-lysosomal pathway indicated that the VML had less colocalization with lysosomes than the control liposome that consisted of a deactivated proton channel. This resulted in effective knockdown of eGFP expression in Hela cells compared to the control. Our mechanistic insights highlight the importance of strategies that actively utilize the endo-lysosomal pH environment to bypass the degradation bottleneck resulting from lysosomal entrapment, and suggest that our construction could serve as a template for target-specific delivery of RNA therapeutics.

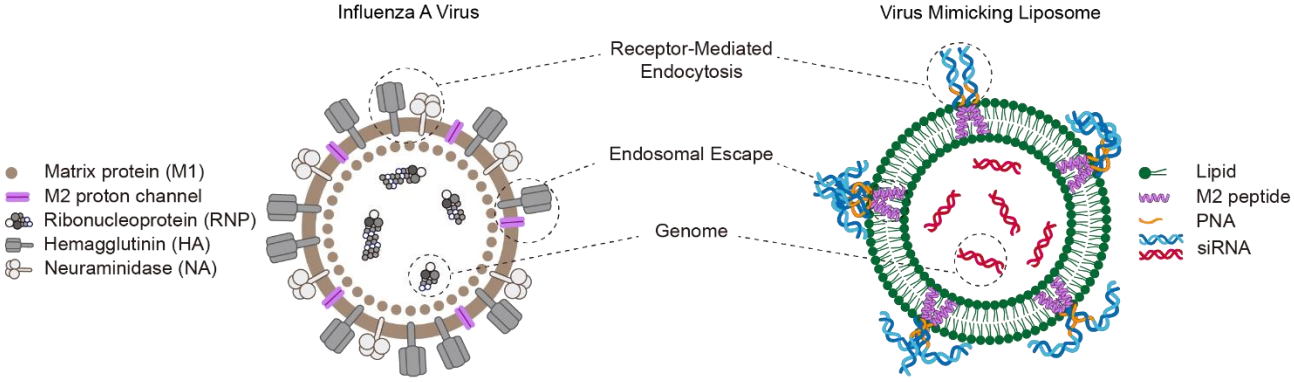

Figure 1. A virus mimicking liposome embedded with M2siRNA complex (M2siRNA@LS). To mimic influenza A virus, (1) siRNA was tethered at the outside of liposome via PNA linker to mediate cellular uptake, (2) M2 proton channel was used to enhance the endosomal escape, and (3) siRNA was encapsulated in liposome to confirm delivery capacity.

References

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