A close shave: How SARS-CoV-2 induces the loss of cilia

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Wang et al. report in this issue (2022. J. Cell Biol. https://doi.org/10.1083/jcb.202108015) that the SARS-CoV-2 protein ORF10 increases the activity of the E3 ligase CUL2ZYG11B, leading to the degradation of multiple ciliary proteins. The resulting loss of cilia may facilitate the spread of SARS-CoV-2 in the respiratory tree.
exclusive, as centrosomes cannot engage in both processes simultaneously. Therefore, the transformed cell lines MRC-5 and NIH3T3 were first serum starved to inhibit cellular division and enable the formation of a primary cilium (Fig. 1 C). This organelle could be detected as a single protrusion positive for a stable microtubule marker, acetylated α-tubulin. Transfection of ORF10 either before or after starvation decreased the percentage of cells carrying a primary cilium, confirming that this viral protein antagonizes both ciliogenesis and cilium maintenance. Overexpression of ZYG11B mimicked the effect of ORF10, while knock-down of ZYG11B attenuated ORF10 effect, consistent with a ZYG11B-dependent inhibition of ciliogenesis by ORF10.

The authors then focused on the role of the ciliary protein Intraflagellar Transport 46 (IFT46), which was profoundly downregulated upon ORF10 expression. IFT46 interacted with ZYG11B but not with ORF10, supporting a model where ORF10 increased the activity of the CUL2\(^{ZYG11B}\) complex towards its substrates. IFT46 overexpression partially rescued ciliogenesis in ORF10 expressing cells, suggesting that IFT46 degradation plays a role in the ORF10-induced ciliogenesis defect. The IFT46 motif recognized by ZYG11B remains to be fully characterized, as the IFT46-ZYG11B interaction did not rely on a canonical mechanism of Gly/N-degron recognition (where ZYG11B binds to an N-terminal glycine).

Figure 1. SARS-CoV-2 ORF10 impairs ciliogenesis by enhancing the activity of the E3 ligase CUL2\(^{ZYG11B}\). (A) The CUL2\(^{ZYG11B}\) RING E3 ligase complex contributes to cellular protein degradation via ubiquitination. (B) Upon SARS-CoV-2 infection, the viral protein ORF10 binds the E3 adapter ZYG11B, increasing the ubiquitination activity of the complex, and inducing the proteasomal degradation of ciliary proteins, including IFT46. (C) ORF10 overexpression in serum-starved NIH3T3 and MRC-5 cells blocks primary cilium biogenesis and maintenance. (D) The lentiviral transfer of ORF10 is sufficient to induce cilia loss in human ACE2 knock-in mice and in primary human nasal epithelial cells, highlighting the role of this viral protein in SARS-CoV-2-mediated cilia disruption.
Rather, Wang et al. found that interaction with ZYG11B required the internal C2 domain of the IFT46 protein. Intriguingly, overexpression of an IFT46 protein lacking the C2 domain still partially rescued ciliogenesis in ORF10-expressing cells, suggesting that cillum recovery did not require a fully functional IFT46 protein nor titration of ZYG11B by excess IFT46. More broadly, whether a specific motif distinct from the Gly/N-degron can target ciliary proteins for ZYG11B-dependent degradation remains to be established. ZYG11B may directly interact with only a subset of the hundreds of ciliary proteins downregulated in the presence of ORF10. Cilia maintenance is highly dynamic, and targeting a master regulator of ciliogenesis can be sufficient to drastically downregulate the expression of multiple ciliary components. SARS-CoV-2 infection of primary ciliated cells was for instance shown to induce an early downregulation of the transcription factor FOXJ1, which is required for cilia formation and maintenance (4). Wang et al. (5) did not observe an effect of ORF10 on FOXJ1 expression, but an effect on another master regulator of ciliogenesis is not ruled out. Interestingly, a role for ORF10 in inducing the autophagic degradation of mitochondria was recently reported (10). The cross-talks between autophagy and ciliogenesis are many (11), raising the possibility that ORF10 may also trigger the autophagic degradation of primary and motile cilia.

A strong point of the study is the demonstration that ORF10 transfer is sufficient to induce cilia loss in vivo (Fig. 1D). Wang et al. (5) used the intranasal inoculation of a lentiviral vector to transfer ORF10 to human ACE2 knock-in mice. Astutely, the authors pseudotyped the ORF10 and control lentivectors with the SARS-CoV-2 spike, ensuring that ORF10 would be transferred to relevant ciliated target cells. Inoculation of the ORF10 vector was sufficient to induce cilia loss in epithelial cells lining the mice trachea. The spike-pseudotyped control vector had no such effect, ensuring that binding of the spike alone was not sufficient to perturb cilia. The authors then verified that infection of the hACE2 mice with authentic SARS-CoV-2 did also induce cilia loss and IFT46 downregulation. Importantly, the authors also found that ORF10 lentiviral transfer could induce cilia loss in a reconstructed human nasal epithelium in vitro, demonstrating the effect of ORF10 in bona fide human multiciliated cells.

This study illustrates yet another way for viruses to highjack the ubiquitin/proteasome pathway. In most instances reported so far, a viral protein targets a specific protein involved in intrinsic/innate defense for E3 recognition and proteasomal degradation. Here, Wang et al. (5) demonstrate that the viral protein ORF10 induces the degradation of an array of ciliary proteins, leading to the disappearance of a whole organelle involved in viral particle clearance.

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