RESEARCH HIGHLIGHT

Proviral role of caspase-6 in coronavirus infections

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Pathogenic human coronaviruses induce apoptosis in infected cells. Caspase-6 activated under apoptotic conditions proteolytically cleaves the nucleocapsid protein into IFN-I antagonists that enhance virus replication, suggesting that caspase-6 has a proviral role in the coronavirus life cycle.

Apoptosis is an important cellular process critical for maintaining host homeostasis. Under certain conditions, such as virus infections, apoptosis of infected cells plays a role in limiting virus replication and spread. However, despite accumulating evidence that coronaviruses hijack specific antiviral cellular pathways, how the virus precisely does so remains elusive.[1] In an article recently published in *Nature*, Chu et al.[2] described an elegant set of experiments dissecting the role of the nucleocapsid (N) proteins of human coronaviruses and a host cysteine-aspartic protease involved in apoptosis, caspase-6, in coronavirus immune evasion and pathogenesis (Fig. 1). The authors reported that highly pathogenic human coronaviruses including Middle-East Respiratory Syndrome Coronavirus (MERS-CoV) and Severe Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the causative agent of COVID-19, induce host cell apoptosis[3] and exploit caspase-6 to facilitate virus replication.[2] The authors demonstrated, using a variety of cell lines, organoids and in vivo models, that pharmacological inhibition or genetic depletion/ablation of caspase-6 significantly diminished virus replication, alleviated pathological changes, and improved survival in infected cells and/or animals. The authors additionally analyzed the mechanism of caspase-6 in promoting virus replication. They discovered that coronavirus N proteins are specifically cleaved by caspase-6 and that the N protein cleavage products potently suppressed type I interferon (IFN-I) expression. In addition, mutant viruses with disrupted putative caspase-6 cleavage sites were no longer sensitive to caspase-6 inhibitor-mediated suppression. Combining all the data, the authors proposed a novel mechanism of immune evasion, illustrating yet another example of how coronaviruses hijack host cell processes for their own agendas.

While Chu et al. identified a role for apoptosis in coronavirus-infected cells, whether uninfected bystander cells also undergo apoptosis has not been investigated. It has been reported that ~1%–2% of cells in the distal airway express ACE2, suggesting that only a limited fraction of cells are susceptible to SARS-CoV-2 infection.[4] However, the number of cells in the alveolar compartment is significantly lower in deceased COVID-19 patients compared to controls.[5] This is consistent with a model in which uninfected bystander cells are depleted after coronavirus infection. If and how uninfected bystander cells are destroyed after coronavirus infection, whether via the extrinsic apoptotic pathway or another form of cell death remains an open question. Understanding the relative importance of infected versus uninfected cell death may contribute to our understanding in pathogenesis. In addition, the suppression of IFN-I expression in infected cells alone does not fully explain the low systemic levels of IFN-I observed in some patients with severe COVID-19,[6] raising the possibility that N protein cleavage products (or other virus products) are released from infected cells, inhibiting IFN-I expression and signaling in nearby, uninfected cells. It will be important to demonstrate the presence of N protein cleavage products in mice so as to support the role of caspase-6 in vivo. It will also be crucial to determine the relevance of caspase-6 in IFN suppression by assessing whether caspase-6 inhibition would equivalently inhibit virus replication in the absence of IFN signaling.

Another key goal is to determine which type of cells undergo apoptosis after infection. Clearance of virus-infected cells is important in limiting virus replication and spread. However, depending on the functions and number of cells being cleared, extensive apoptosis, despite being critical in controlling infection, also results in cell loss and impaired tissue function. In the case of SARS-CoV-2, a reduced number of alveolar cells in the lungs and lymphopenia are often observed in patients with severe COVID-19.[5] Moreover, several reports indicate that alveolar type II (AT2) and to a lesser extent, alveolar type I (AT1) cells, important for gaseous exchange, are susceptible to SARS-CoV-2 infection,[4] raising the possibility that the diminished number of alveolar cells may contribute to pathogenesis. In addition, lymphocytes, myeloid cells and renal cells were shown to undergo apoptosis after coronavirus infection, with no evidence of productive infection.[7,8] Another possibility is that extrinsic apoptosis of uninfected cells is directly induced by viral proteins or cytokines such as tumor necrosis factor released from disintegrating infected cells.[8,9]

Chu et al. identified a specific mechanism whereby caspase-6 promotes virus replication under apoptotic conditions.[2] In the intrinsic apoptotic pathway, caspase-9 and caspase-3 are required for activating caspase-6.[10] However, inhibition of caspase-3 did not impair virus replication, suggesting that other caspases compensated for caspase-3 inhibition. Caspase-7 has been reported to substitute for caspase-3 when caspase-3 is disabled.[10] Also, when the authors blocked the activity of caspase-9 or caspase-10, a significant but less robust reduction in replication was observed.[2] This suggests that other caspases may also have a role in promoting virus replication, possibly through distinct mechanisms. Understanding how caspases enhance virus replication may inform design of a novel set of antivirals.

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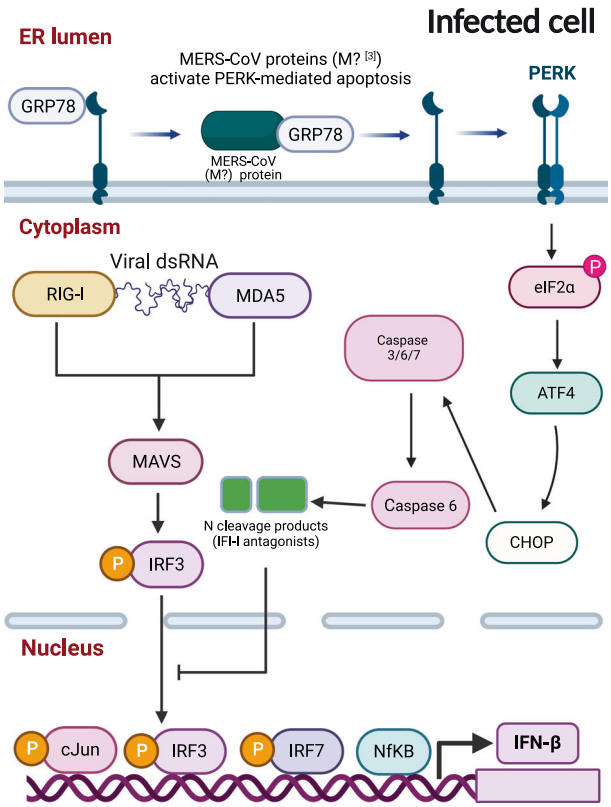

Fig. 1 Proviral role of caspase-6. MERS-CoV protein, possibly the M protein, sequesters 78-kDa glucose-regulated protein (GRP78) and releases protein kinase R-like endoplasmic reticulum kinase (PERK) for activating PERK-mediated apoptosis.[3] Caspase-6 is activated under apoptotic conditions, presumably by the PERK pathway and cleaves the N protein into IFN-I antagonists that impede IFN regulatory factor 3 (IRF3) translocation into the nucleus for inducing IFN-I expression.[2] The figure was created with BioRender.com.

REFERENCES

1. Gassen, N. C. et al. *Nat. Commun.* **12**, 3818 (2021).
2. Chu, H. et al. *Nature* https://doi.org/10.1038/s41586-022-05148-4 (2022).
3. Chu, H. et al. *Sci. Adv.* **7**, 25 (2021).
4. Hou, Y. J. et al. *Cell* **182**, 429–446.e14 (2020).
5. Melms, J. C. et al. *Nature* **595**, 114–119 (2021).
6. Hadjadj, J. et al. *Science* **369**, 718–724 (2020).
7. Yeung, M. L. et al. *Nat. Microbiol.* **1**, 16004 (2016).
8. Chu, H. et al. *J. Infect. Dis.* **213**, 904–914 (2016).
9. Ren, Y. et al. *Front. Cell. Infect. Microbiol.* **11**, 706252 (2021).
10. Cullen, S. P. & Martin, S. J. *Cell Death Differ.* **16**, 935–938 (2009).

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ADDITIONAL INFORMATION

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