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Research Highlight

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SARS-CoV-2 ORF7a protein blocks virus clearance by regulating autophagy

Huimei Liu†, Qin Li†, and Lanfang Li*

Institute of Pharmacy and Pharmacology, Hunan Provincial Key Laboratory of Tumor Microenvironment Responsive Drug Research, Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study, Hengyang Medical School, University of South China, Hengyang 421001, China

†These authors contributed equally to this work.

*Correspondence address. Tel: +86-17674708255; E-mail: llanfang6@126.com

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Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a serious public health threat worldwide. The most common clinical symptoms of COVID-19 include fever, cough, anorexia, sputum production, expectoration, shortness of breath, fatigue, *etc*. Conventional therapeutics such as antiviral drugs, vaccines, anti-SARS-CoV-2 antibody treatments, and convalescent plasma therapy are currently under extensive research and clinical trials for the treatment of COVID-19 [1].

With the ongoing pandemic of COVID-19, further investigation into the high infectivity and pathogenicity of SARS-CoV-2 and exploration of effective anti-SARS-CoV-2 therapeutic drugs are needed. The 5′-terminus of the SARS-CoV-2 genome has two overlapping ORFs (ORF1a and ORF1b), which encode 16 non-structural proteins (NSPs). The 3′-terminus of the SARS-CoV-2 genome contains spike (S), membrane (M), envelope (E) and nucleocapsid (N) structural proteins and a number of genus-specific accessory proteins (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9, and ORF10) (Figure 1A). Recently, Hou *et al*. [2] revealed that ORF7a promotes autophagosome accumulation in multiple cells. More importantly, the ORF7a protein in SARS-CoV-2 initiates autophagy and limits autophagosome-lysosome fusion by degrading SNAP29, thereby promoting viral replication.

Autophagy, which is an evolutionarily conserved catabolic process of protein and organelle degradation, plays an important role in maintaining cellular homeostasis [1]. A large number of studies have indicated that autophagy is categorized into micro-autophagy, macroautophagy and chaperone-mediated autophagy. Autophagy is strongly associated with viral infections. As a defense strategy of organisms, autophagy can be triggered to antagonize viral infections by delivering cytoplasmic virions or viral components to lysosomes for degradation. In addition, degradation also promotes the inflammatory response, antigen presentation, and clearance for pathogen recognition. However, recent studies have shown that some viruses inhibit or evade autophagy, whereas some viruses even hijack autophagy. With the onset of the global COVID-19 epidemic, the relationship between autophagy and SARS-CoV-2 has attracted much more scientific attention [3].

Recently, Hou *et al*. [2] confirmed that the expression of LC3-II is dramatically increased but the expression of SQSTM1 is not decreased in SARS-CoV-2-infected Caco2, Vero-E6 and Calu-3 cells, which leads to the accumulation of autophagosomes. Furthermore, they revealed that SARS-CoV-2 infection-induced accumulation of autophagosomes enhances viral replication and that the inhibition of autophagy by using 3-MA and siRNA-ATG7 could inhibit intracellular and extracellular viral loads. They further verified that ORF7a, a protein of SARS-CoV-2, leads to the accumulation of autophagosomes but impairs the fusion of autophagosomes with lysosomes. The dual roles of ORF7a contribute to SARS-CoV-2-induced incomplete autophagy. They further investigated the detailed molecular mechanism of ORF7a-stimulated incomplete autophagy. On the one hand, ORF7a protein induces the initial stages of autophagy by suppressing the AKT-mTOR-ULK1-mediated pathway. On the other hand, ORF7a activates CASP3, which can cleave SNAP29, and then autophagosome-lysosome fusion is impaired. SNAP29 can trigger degradative autophagy to inhibit viral replication. However, ORF7a-induced inhibition of SNAP29 results in viral replication. Regarding the relationship between SARS-CoV-2 and autophagy, Hou *et al*. showed that SARS-CoV-2 infection suppresses the AKT-mTOR-ULK1 pathway and triggers the accumulation of autophagosomes. These accumulated autophagosomes cannot fuse with lysosomes because the SARS-CoV-2 ORF7a protein inhibits the role of SNAP29 and hijacks autophagic flux, which finally results in the accumulation of viruses in autophagosomes and the blocking of autophagy-induced viral clearance [2] (Figure 1B).

Hou *et al*. [2] also revealed that ORF7a, which is a genus-specific accessory protein of SARS-CoV-2, contributes to the accumulation

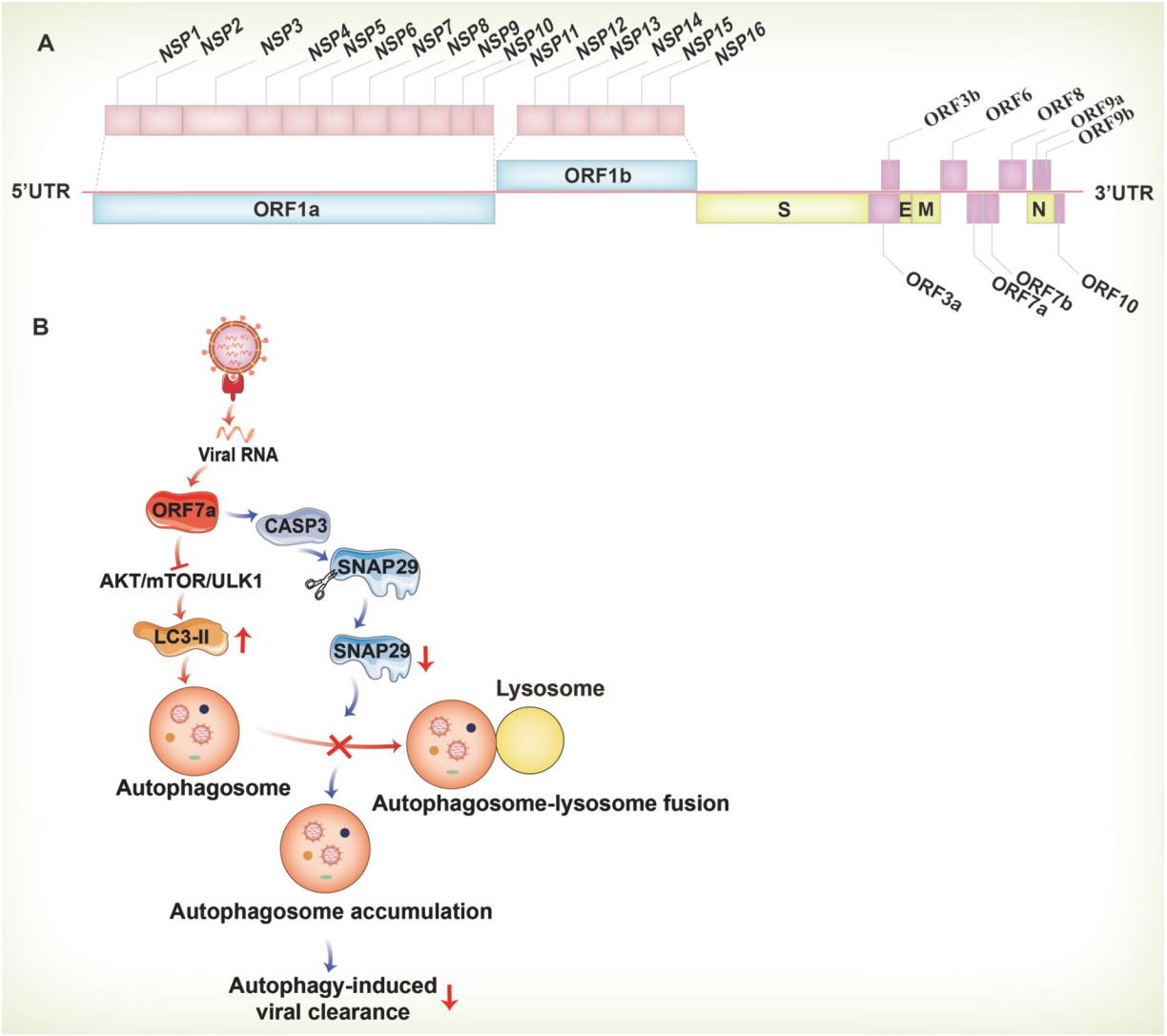

Figure 1. The structure of SARS-CoV-2 ORF7a protein and the mechanism of its role in regulating autophagy (A) Schematic of the SARS-CoV-2 genome structure. ORF, open reading frame; S, spike structural protein; M, membrane structural protein; E, envelope structural protein; N, nucleocapsid structural protein; NSP1-NSP16, nonstructural proteins. (B) Dual mechanism by which the SARS-CoV-2 ORF7a protein regulates autophagy. ORF7a can suppress the AKT-mTOR-ULK1-mediated pathway and promote the expression of LC3-II, which induces the initial stages of autophagy. In addition, ORF7a activates CASP3, which cleaves SNAP29, impairing autophagosome-lysosome fusion and resulting in damaged autophagy-induced virus clearance. CASP3, caspase-3; SNAP29, synaptosome associated protein 29.

of autophagosomes and hijacks autophagic flux. Besides ORF7a, there are a number of genus-specific accessory proteins. The relationships between these accessory proteins and autophagy are obscure. The SARS-CoV-2 ORF3a protein blocks the maturation of autophagosomes into autolysosomes, which is similar to ORF7a [4]. Moreover, when cells are inoculated with SARS-CoV-2, the expression profile of viral genes, the interaction between viral proteins and host and the expression profile of host genes might be different. The continued evolution of SARS-CoV-2 has also led to the emergence of several new subvariants [5]. Therefore, further studies are required to determine whether multiple proteins of SARS-CoV-2 and subvariants have a balanced mechanism to promote the accumulation of autophagosomes, which is conducive to viral replication.

It is well known that starvation-induced autophagy is thought to randomly degrade cellular components. However, under certain circumstances, autophagy selectively degrades specific targets for quality control, which is named selective autophagy, such as mitophagy, pexophagy, endoplasmic reticulum (ER)-phagy, ribophagy, lysophagy, golgiphagy and nucleophagy [6,7]. Autophagy-induced viral clearance might also belong to selective autophagy. It has also been reported that in SARS-CoV-2-infected cells, the newly synthesized S protein in the ER is transported first to the Golgi apparatus and then from the Golgi apparatus to the ER-Golgi intermediate compartment, resulting in specific motif formation at the C-terminal end. These studies indicated that major events of SARS-CoV-2 infection are associated with the ER and Golgi apparatus [8]. Both the ER and Golgi apparatus can be selectively degraded by ER-phagy and golgiphagy, respectively. However, the role of SARS-CoV-2 infection in selective ER-phagy and golgiphagy has not been described.

Increasing evidence supports that viruses, including SARS-CoV-2,

can induce the initiation of autophagy, which is a powerful tool that host cells use to defend against viral infection. However, SARS-CoV-2 subverts and highjacks the flux of autophagy by inhibiting autophagosome fusion with lysosomes, which contributes to the escape elimination of SARS-CoV-2. To date, two distinct approaches have been explored for useful therapeutic drugs: prevention of SARS-CoV-2 entry into host cells and suppression of virus replication inside host cells. We consider that new molecular drugs that are involved in the regulation of autophagy-induced viral clearance could be combined with drugs that have been used clinically. Exploring novel molecular drugs that can promote autophagic flux and enhance autophagy-induced viral clearance might become a new strategy for treating COVID-19.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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