

REVIEW

Cell death mechanisms involved in cell injury caused by SARS-CoV-2

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Abstract

Coronavirus disease 2019 (Covid-19) is an emerging novel respiratory infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that rapidly spread worldwide. In addition to lung injury, Covid-19 patients may develop extrapulmonary symptoms, including cardiac, liver, kidney, digestive tract, and neurological injuries. Angiotensin converting enzyme 2 is the major receptor for the entry of SARS-CoV-2 into host cells. The specific mechanisms that lead to cell death in different tissues during infection by SARS-CoV-2 remains unknown. Based on data of the previous human coronavirus SARS-CoV together with information about SARS-CoV-2, this review provides a summary of the mechanisms involved in cell death, including apoptosis, autophagy, and necrosis, provoked by severe acute respiratory syndrome coronavirus.

KEYWORDS

cell death, pathways, Sars-CoV-2

1 | INTRODUCTION

Covid-19 (Coronavirus disease 2019) is a new emerging respiratory infectious disease caused by severe acute respiratory syndrome

coronavirus 2 (SARS-CoV-2).¹ The virus was discovered in late December 2019 in Wuhan, Hubei Province, China, and quickly spread around the world.^{2,3} On 30 January 2020, WHO (World Health Organization) declared this outbreak a Public Health Emergency of

Abbreviation: ACE2, Angiotensin converting enzyme 2; AIF, Apoptosis inducing factor; AMBRA1, Activating molecule in BECLIN-regulated autophagy 1; ARDS, Acute respiratory distress syndrome; ASK, Apoptosis signal-regulating kinase; ATG, Activating transcription factor; β Cov, Betacoronavirus; Bcl-2, B-cell lymphoma; Bcl-XL, B-cell lymphoma extra-large; CCL2, C-C motif chemokine ligand 2; CD, Cluster of differentiation; CHOP, C/EB homologous protein; COPD, Chronic obstructive pulmonary disease; Covid-19, Coronavirus disease 2019; CoVs, Coronaviruses; CQ, Chloroquine; CTLA4, Cytotoxic T-lymphocyte associated protein 4; CypD, Cyclophilin D; Cyt c, Cytochrome c; DAMPS, Damage-associated molecular patterns; DFCEP1, Double FYVE-containing protein 1; DNA, Deoxyribonucleic acid; E, Envelope protein; EGFR, Epidermal Growth factor receptor; ER, Endoplasmic reticulum; FADD, Fas associated via death domain; FASL, Fas Ligand; FKHL1, Forkhead transcription factor; GAS6, Growth arrest specific 6; GRP78, Glucose-regulated 78; GRP94, Glucose-regulated 94; GSDM, gasdermin D-regulated; HCoV, Human coronavirus; HCQ, Hydroxychloroquine; IL, Interleukin; INF, interferon; IRE-1, inositol-requiring enzyme 1; IRF, Interferon regulatory factor; ISG, Interferon-stimulated genes; JNK, c-Jun N-terminal Kinase; LDH, Lactate dehydrogenase; MAPK, Mitogen activated Protein Kinase; MERS-CoV, Middle East respiratory syndrome coronavirus; MERTK, MER proto-oncogene, tyrosine Kinase; MHV, Mouse hepatitis virus; MLKL, Mixed-lineage Kinase domain-like pseudo Kinase; MPT, Mitochondrial permeability transition; MPTP, Mitochondrial permeability transition pore; NF κ B, Nuclear factor kappa B; NSP, non-structural protein; ORF, Open reading frame; PDK1, 3-phosphoinositide-dependent protein kinase-1; PERK, Protein Kinase RNA-like endoplasmic reticulum Kinase; PKB, Protein Kinase B/Akt; PtdIns3KC3, Phosphatidylinositol 3-Kinase class 3; RIPK, Receptor-interacting protein Kinase; RNA, Ribonucleic acid; S, Spike protein; SARS-CoV-2, Severe-acute respiratory syndrome coronavirus 2; SIRT4, Sirtuin 4; TFEB, Transcription factor EB; TGF- β , Transforming growth factor beta; TLR4, Toll-like receptor 4; TNF, Tumor necrosis factor; TYRO3, Tyrosine-protein Kinase 3; UBC, Ubiquitin C; WHO, World Health Organization.

International Concern.⁴ As of 16 August 2021, the disease has affected more than 206, 693, 357 million people, being responsible for 4,352, 488 deaths.⁵

Coronaviruses (CoVs) are enveloped, positive-sense, single-stranded RNA viruses. They are genetically classified into four major genera: *Alphacoronavirus*, *Betacoronavirus* (β CoV), *Gammacoronavirus*, and *Deltacoronavirus*. Coronaviruses possess the largest RNA genomes (27–32 kb) among the RNA viruses, the 5' and 3' ends of coronaviruses genomes contain short untranslated regions. For the coding regions, CoV viral genome encodes four major structural proteins: the spike (S) protein, the envelope (E) protein, the membrane (M) protein, and the nucleocapsid (N) protein. Sars-CoV-2 is classified in the genus β CoV, like SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), with evidence indicating that the novel CoV is of bat-origin. In addition, next-generation sequencing shows that SARS-CoV-2 shares 79% sequence identity to SARS-CoV and 50% to MERS-CoV.^{6–12}

The clinical manifestations of Covid-19 include fever, cough, myalgia or fatigue, dyspnea and radiographic evidence of pneumonia.^{13,14} The diffuse alveolar damage and acute respiratory failure are the main features of Covid-19.^{13,15} Studies suggest that previous comorbidities such as hypertension, diabetes, chronic obstructive pulmonary disease (COPD) and other cardiovascular diseases are associated with severe cases of the disease.^{16–19} The fatal cases present complications as acute respiratory distress syndrome (ARDS), acute cardiac injury, acute kidney injury, and shock, eventually followed by multiple organ failure.^{16,20,21}

The cell death mechanisms associated with cell injury caused by the SARS-CoV-2 are not well understood nor established. Previous studies have shown that different apoptosis mechanisms are activated in coronavirus infection^{22–34}. Previous finds from SARS CoV and MERS studies can be used as reference to investigate molecular cell death mechanisms involved in SARS-CoV-2 infection. Therefore, two reviewers conducted independent literature research at Medline via Pubmed up to March 30 using the following strategy: “Severe Acute Respiratory Syndrome Virus” OR “SARS-Related Coronavirus” OR “SARS-CoV” OR “SARS Coronavirus” OR “Coronavirus, SARS” OR “Severe acute respiratory syndrome-related coronavirus” OR “Severe acute respiratory syndrome related coronavirus” OR “SARS-Associated Coronavirus” OR “Coronavirus, SARS-Associated” OR “SARS Associated Coronavirus”) AND (“cell death” OR “apoptosis” OR “Autophagic cell death” OR “immunogenic cell death” OR “regulated cell death”. A total of 370 studies were identified and then filtered by title and Abstract. After reviewing the remaining articles, 33 studies were included in this review. The two authors (Marillya Morais da Silva and Priscilla Stela Santana de Oliveir) resolved disagreements by consensus and a third author (Michelly Cristiny Pereira) did the final revision.

2 | CORONAVIRUS AND APOPTOSIS

Apoptosis is a process of programmed cell death, morphologically characterized by cellular shrinkage, condensation and margination of

chromatin, a dense cytoplasm with tightly packed organelles and the formation of apoptotic bodies. Apoptosis is a natural phenomenon that occurs during growth, ageing, and cellular homeostasis. It may also happen as a result of infections or cell injury.^{35–38}

The mechanisms responsible for apoptosis can be grouped into two main pathways: the extrinsic pathway of apoptosis and the intrinsic pathway. In the extrinsic pathway, transmembrane receptor-mediated interactions initiate the process of apoptosis, the sequence of events that integrates this pathway can be demonstrated by Fas Ligand (FasL)/Fas models. The binding of Fas ligand to Fas receptor results in the binding of the adapter protein FADD. FADD then associates with procaspase-8 via dimerization of the death effector domain, when caspase-8 is activated, the execution phase of apoptosis is triggered. While in the intrinsic way, stimuli cause changes in the inner mitochondrial membrane that results in an opening of the mitochondrial permeability transition (MPT) pore, loss of the mitochondrial transmembrane potential and release of two main groups of normally sequestered pro-apoptotic proteins. The first group includes Cytochrome c (CytC), a molecule that binds and activates Apaf-1 and procaspase-9, forming the apoptosome, which later results in caspase 9 activation. Both paths converge to an execution path, where Caspase-3 is activated by any of the initiator caspases (caspase-8, caspase-9, or caspase-10), activating caspase 3 results in events like endonuclease activation, degradation of chromosomal DNA, protease activation and degradation of nuclear and cytoskeletal proteins, culminating in the cytomorphological changes characteristic of apoptosis.^{35,39–42}

2.1 | Apoptosis induction by SARS-CoV open reading frames

Coronaviruses can cause apoptosis via a variety of signaling pathways. SARS-CoV and SARS-CoV-2 have two long open reading frames, orf1a and orf1ab, encoding polyproteins 1a and 1ab. The polyproteins are cleaved by viral proteases into 16 non-structural proteins, Nsp1–Nsp16.⁴³ Genome contains eight accessory genes whose open reading frames are interspersed among the structural genes; two between S and E genes (ORFs 3a and 3b), five are located between the M and N genes (6, 7a, 7b, 8a, 8b) and one within the N gene (9b) (Figure 1).⁴⁴ Schaecher, Scott R., and Andrew Pekosz has summarized the functions and the subcellular localization of the SARS-CoV accessory proteins⁴⁵. ORF3a is localized mostly in Golgi and plasma membrane, data propose that it has a role in virus release and lifecycle of SARS-CoV.^{46–48} On the other hand, ORF3b is localized in nucleolus and mitochondria, and induces cell death and prevents interferon- β (IFN- β) production.^{49–52}

The ORF6 protein is subcellular, found in ER and Golgi, and packaged into virus particles and incorporated into virus-like particles.^{43–54} Other studies suggest that ORF6 is also related to virus assembly or replication processes and antagonizes IFN- β production.^{55,56} Overexpression of ORF6 of SARS-CoV induced apoptosis via caspase-3-mediated, dependent pathways, and caspase-3

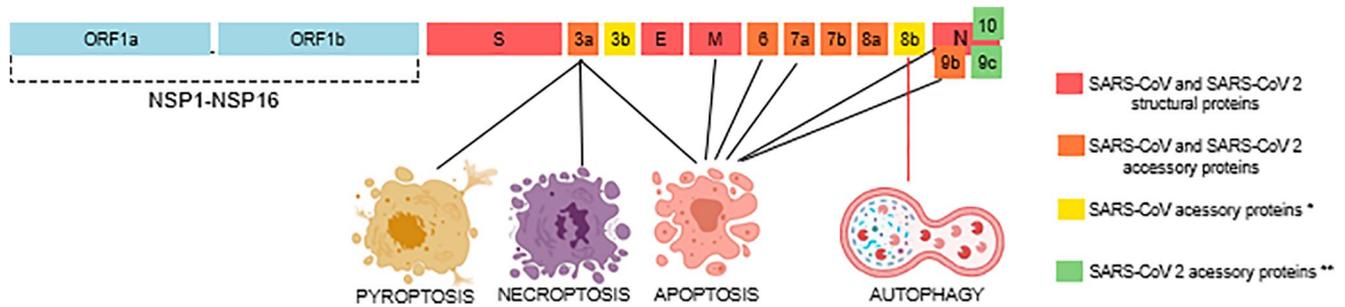


FIGURE 1 Illustration of the genome of severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 showing the location of structural proteins, open reading frames (orf1a and orf1ab), accessory proteins which they are known to trigger different forms of cell death. *ORF3b and ORF8b is only present in SARS-CoV. **ORF10 is only present in SARS-CoV-2

inhibitor and c-Jun N-terminal kinase (JNK) inhibitor blocked ORF-6 induced apoptosis. The protein level of ER chaperon protein, GRP94, was up-regulated which suggests that overexpression of ORF-6 could induce apoptosis via the ER-stress pathway too.^{57,58}

Gene 7 encodes two accessory proteins, ORF7a and ORF7b. ORF7a is subcellular localized in the Golgi region of transfected and infected cells and its specific function remains unknown.⁵⁹ ORF7a may play a role in different processes such as apoptosis induction, activation of signaling pathways and others.^{21,60} ORF7b is also localized in the Golgi region using cDNA transfected and SARS-CoV infected cells.^{59,61} ORF7b is associated with intracellular virus particles and with apoptosis.^{54,55} Furthermore, Tan et al. suggests SARS-CoV 7a protein triggers apoptosis by interfering directly with the function of Bcl-XL, a prosurvival member of the Bcl-2 family. The results showed that overexpression of Bcl-XL, blocks 7a-induced apoptosis, implying that the mechanism behind apoptosis induced by the 7a protein is at the level of or upstream from the Bcl-2 family.⁶²

Gene 8 also contains two ORFs. ORF8a is found in the cytoplasm and mitochondria and is reported to have proapoptotic effects.^{63,64} ORF8b is localized in cytoplasm and its function is still a mystery. A study has suggested a potential role for ORF8b in modulating degradation or stability of the SARS-CoV E protein.⁶⁵ Lastly, ORF9b is localized to the ER region of transfected cells and may interact with viral proteins nsp8, nsp14, and ORF7b.⁶⁶ In addition, it was proposed that 9b protein is associated with nucleocytoplasmic export linked apoptosis. The study showed that the 9b protein, known to be localized in the extra-nuclear region, was also present in the nucleus, and blocking 9b nuclear export led to caspase-3 dependent apoptosis of the host cells.⁶⁷

Another open reading frame of SARS-CoV-2 was annotated, the ORF9c.⁶⁸ Dominguez et al. has proposed that ORF9c is a membrane-associated protein that suppresses antiviral responses in cells. ORF9c enables cells to escape from immune surveillance through reduced HLA abundance and antigen presentation, also slowing cell replication.⁶⁹ SARS-CoV-2 has another accessory protein, ORF10 is located within the N gene, evidence shows that ORF10 might bind to CUL-ZYG11B complex and hijack its ubiquitination of restriction factors.⁴³

Some of these accessories proteins trigger apoptosis by different mechanisms. Tan et al. propose that 7a protein of SARS-CoV can

induce apoptosis when overexpressed, as evidenced by an increase in caspase-3 protease activity, a hallmark of apoptosis.²³ Kopecky-Bromberg et al. have described that the 7a protein has the ability to inhibit cellular translation and activate p38 Mitogen Activated Protein Kinase (MAPK); this may be the mechanism that leads to apoptosis induction.⁶⁰

The 3a protein participates in several signaling pathways that lead to apoptosis. Padhan et al. described that SARS-CoV 3a protein activates the mitochondrial death pathway through p38 MAP kinase activation.²² Activation of p38 kinase, leads to upregulation of p53, which can increase nuclear transcription of the Bax gene, promoting cytoplasmic oligomerization of the Bax protein. Bax multimers insert in the mitochondrial membrane, provoke loss of potential and release of CytC, which promotes formation of the apoptosome and activation of caspase-9.⁷⁰

Other in vitro studies confirmed the role of orf-3a in inducing apoptosis. Law et al. examined the expression levels of Bcl-2 family proteins and caspase-8, which mediate the intrinsic and extrinsic pathway of apoptosis, respectively. Cleavage of pro-caspase-8 was increased in 3a-transfected Vero E6 cells, indicating that the protein activates apoptosis by its extrinsic pathway.⁷¹ In a more recent study, Ren et al. evaluated the effects of SARS-CoV-2 ORF-3a protein in three cell strains: HEK293T cell line, a human embryonic kidney cell line; HepG2, a human liver cancer cell line; and VeroE6, the Vero lineage derived from kidney epithelial cells extracted from an African green monkey. Results imply that after transfection, SARS-CoV-2 ORF-3a protein can induce apoptosis by activating caspase-8, which when activated cleaves BH3-interacting domain death agonist (Bid) to tBid, which in turn induces the release of CytC, resulting in the formation of apoptosome and cleavage of caspase-9.⁷²

Furthermore, 3a protein can form ion channels on membranes. Chan et al. reported that 3a induced apoptosis is related to the disturbance of intracellular ion homeostasis, implying that perturbation of intracellular ion flux could be one of the SARS-CoV pathogenic mechanisms.³¹ The 3a protein also causes ER stress by activation of the protein kinase RNA-like endoplasmic reticulum kinase (PERK) pathway, which enhances protein folding in the ER as described by Minakshi et al. When it activates only the PERK, but not the Inositol-Requiring Enzyme 1 and Activating Transcription Factor

6 pathways during the unfolded protein response (UPR), the 3a protein protects itself and other viral proteins from ER-associated protein degradation. Prolonged PERK activation would lead to apoptosis. The activation of downstream effectors such as activating transcription factor 4 and C/EBP homologous protein may be yet another mechanism through which the 3a protein can be apoptotic.²⁸

ORF3a is involved with the formation of intracellular vesicles, but the mechanism of membrane rearrangement and vesicle formation remains poorly understood. Freundt et al. reported that ORF 3a is necessary for fragmentation of the Golgi apparatus and suggested that this could be explained by the 3a protein ability to disturb Golgi regulator protein, Arf1, function.⁷³ They also showed that the 3a protein accumulates and localizes to vesicles containing markers for late endosomes and its overexpression is sufficient to determine cell death.

2.1.1 | Apoptosis induction by SARS-CoV structural proteins

Coronaviruses have four major structural proteins. The membrane (M) protein and the nucleocapsid (N) protein can trigger apoptosis by several mechanisms. The SARS-CoV N protein has as primary function the packaging of the genomic RNA in a protective covering.⁷⁴ Surjit et al. discovered that the N protein also has the pro-apoptotic ability to up-regulate JNK and p38 MAPK activities. Expression of the protein also down-regulates the levels of phospho (p)-Akt and Bcl-2, and activates caspases 3 and 7, which leads to apoptosis.²⁹

Some of the SARS-CoV M protein roles include mediating nucleocapsid incorporation into the newly formed virions, regulating viral replication, and packing genomic RNA into viral particles.^{37,38} M protein also has a role in induction of apoptosis in SARS-CoV infected cells. Chan et al. observed that M protein over-expression induced apoptotic cell death with nuclear condensation and release of mitochondrial CytC.³⁸ Beyond that, over-expression of the M protein also caused down-regulation of Akt phosphorylation, which would further reduce the cell survival signal, consequently, leading to induction of apoptosis.

Zhang et al. transfected COS-1, Huh-7 and HepG2 cells with pCDNA3.1(-)/his-myc vector containing the SARS coronavirus N, M and S genes.⁷⁵ The COS-1 lineage, a fibroblast-like cell line derived from monkey kidney tissue, when transfected SARS coronavirus N protein increased intracellular reactive oxygen species and CytC release, with caspase-3, 9 activation and PARP cleavage, leading to apoptosis. While SARS coronavirus M, S protein cannot induce apoptosis in COS-1, HepG2 and Huh-7, SARS coronavirus N protein also did not induce apoptosis in HepG2 and Huh-7. These findings imply that coronavirus proteins behave uniquely in different cell types.

A more recent study proposes that the M-protein compromises the protein kinase B/Akt (PKB) cell survival-signaling pathway through interfering with its upstream activator 3-phosphoinositide-dependent protein kinase-1(PDK1). Tsoi et al. described that the

M-protein can interact with PDK1, PDK1 mediates PKB/Akt phosphorylation.²⁷ PDK1 interaction with the M-protein reduces PKB/Akt phosphorylation. The forkhead transcription factor (FKHRL1) regulates FasL expression and its phosphorylation is regulated by PKB/Akt activity. When phosphorylated, FKHRL1 is held in cytoplasm, where it cannot mediate FasL expression. With the activity of PKB/Akt compromised, FKHRL1 translocates to the nucleus and induces FasL expression. Increased FasL leads to the activation of caspase 8, an apoptotic mediator. Furthermore, PKB/Akt also regulates ASK (apoptosis signal-regulating kinase), down-regulated PKB/Akt activity results in reduction in the level of phosphorylated ASK, which leads to caspase 9 activation.

The mechanisms that leads to apoptosis triggered by structural and accessory proteins of SARS-CoV is summarized in Figure 2.

2.1.2 | Other coronavirus mechanisms that lead to apoptosis

Besides the structural and accessory proteins, coronavirus can induce apoptosis by other mechanisms. Chu et al. suggested that MERS-CoV infected T cells from the peripheral blood and from human lymphoid organs, including the spleen and the tonsil induced apoptosis in T cells by the activation of caspases 3, 8 and 9, triggering activation of the extrinsic and intrinsic apoptosis pathways.⁷⁶ Bellesi et al. observed increased CD95 (Fas) expression in peripheral blood T lymphocytes in COVID-19 patients. It suggests that CD95 induced apoptosis could be a possible mechanism for COVID-19-induced lymphopenia⁷⁷. CD95 is a cell surface protein that can mediate apoptosis bound to its ligand, CD95L. CD95L is mainly expressed in activated T lymphocytes and natural killer cell.⁷⁸

Yeung et al. revealed that MERS-CoV induces apoptosis in kidney and lung by upregulating the mothers against decapentaplegic homolog 7—a homolog of the drosophila gene (Smad7) and the fibroblast growth factor 2 (FGF2) proteins.⁷⁹ Other studies have also linked smad7 with apoptosis in renal disease pathogenesis by inducing apoptosis in mesangial cells and podocytes.^{80,81} Smad7 also induced apoptosis in hepatocarcinogenesis in vitro through the attenuation of NFκB and TGFβ signaling.⁹⁸ In its turn, FGF2 induced podocyte injury and glomerulosclerosis in rat model.⁸²

As has been shown, depending on the cell, the tissue in its microenvironmental context, the SARS family virus can induce different apoptosis mechanisms. Favreau et al. suggested that some HCoV strains can invade the central nervous system, where they induce neuronal programmed cell death cyclophilin D (CypD) dependent and potentially caspase dispensable.⁸³ CypD is responsible for modulation of mitochondrial permeability transition pore (mPTP) in various types of cell death.⁸⁴ When it opens, mPTP allows the release of proapoptotic factors such as CytC and apoptosis-inducing factor (AIF).⁸⁵ AIF translocates to the nucleus and promotes high-molecular-weight DNA fragmentation and chromatin condensation.⁸⁶ However, it is still poorly understood which cellular proteins interacts with CypD to promote mPTP formation.

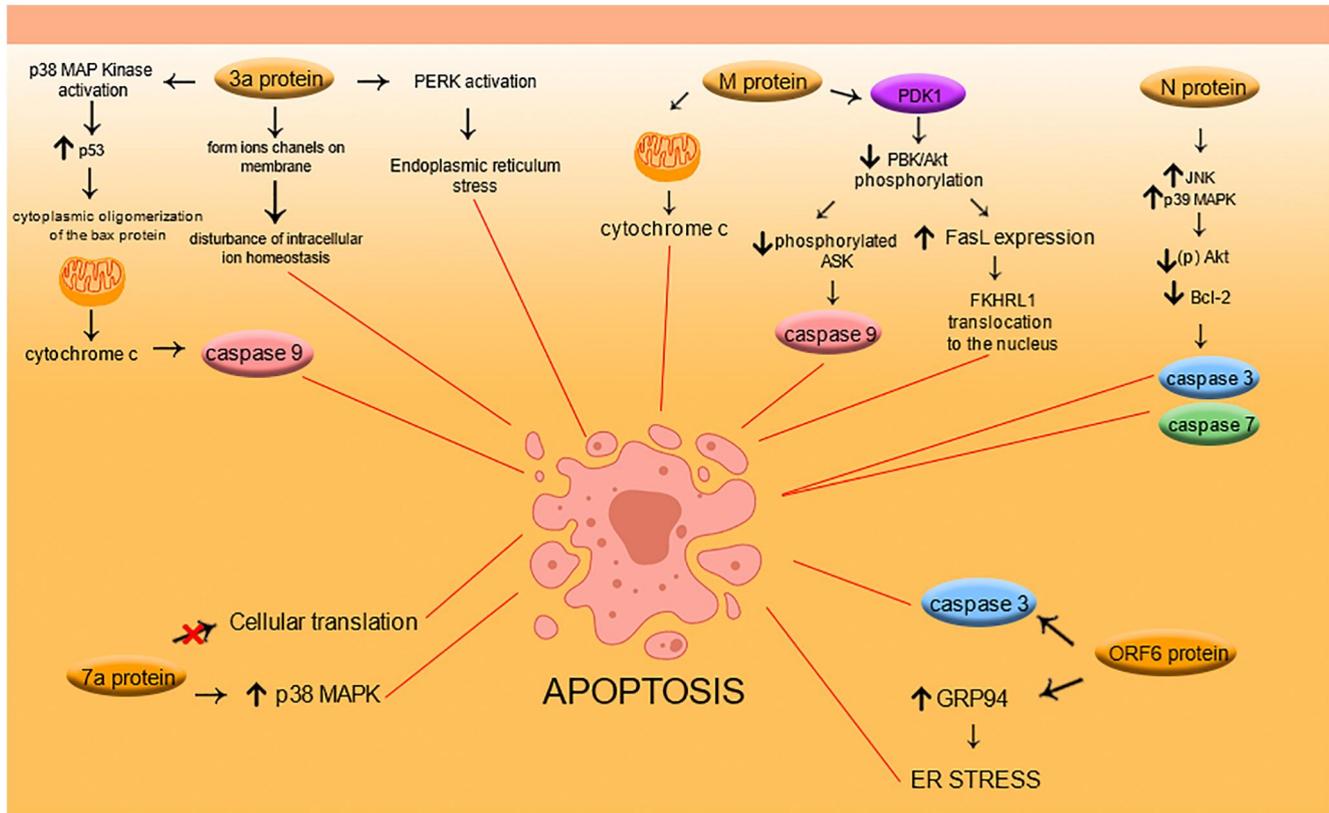


FIGURE 2 Summary of the mechanisms that lead to apoptosis triggered by the structural and accessory proteins of severe acute respiratory syndrome coronavirus (SARS-CoV)

3 | CORONAVIRUSES AND AUTOPHAGY

Autophagy is a cytoprotective tool that enables cellular decomposition and recycling. This process is essential for normal cellular development and growth, having a role in regulating the balance between protein synthesis and degradation, aging, cancer, neurodegenerative disorders, and lysosomal disorders. It can be initiated by several stimuli such as nutrient deprivation, oxidative stress, hypoxia, protein aggregates and toxic molecules to mitigate stress.^{36,87–89} Autophagosomes are double membrane vesicles that engulf part of the cytoplasm that contains long-lived proteins, pathogens, and damaged organelles. To complete the self-digesting mechanism, the autophagosome fuses with endosomal and/or lysosomal vesicles in a maturation process, resulting in autolysosomes.^{90–92}

Previous studies using mouse hepatitis virus as a prototype, showed that (βCoV) induce autophagy related 5 (ATG5)-dependent autophagy and ATG5-dependent autophagosome formation via nsp6.⁹³ NSP6 is also present in other CoVs, including SARS-CoV-2, but intriguingly, other researchers reported that this protein limits further autophagosomal expansion, compromising autophagic delivery of viral components to lysosomes.⁹⁴ Autophagy-related proteins are responsible for autophagy regulation and ATG5 has important roles in canonical and non-canonical autophagy. It is indispensable for autophagic vesicle formation, during the canonical autophagy, ATG5 binds with ATG12 and ATG16 forming a complex

that contributes to autophagosome maturation.⁹⁵ The canonical formation of autophagosomes consists of four steps: 1. initiation; 2. nucleation; 3. elongation and closure; 4. recycling. Each of them involves the activity of ATG proteins, as well as non-ATG proteins such as phosphatidylinositol 3-kinase class III (PtdIns3KC3), p150, activating molecule in BECLIN1-regulated autophagy 1 (AMBRA1) and double FYVE-containing protein 1 (DFCP1). While the non-canonical autophagy can bypass some of these steps, the formation of the double-membraned autophagosome does not require the hierarchical intervention of all of the ATG proteins; the alternative mechanisms are currently under debate.⁹⁶

Moreover, the viral membrane-anchored papain-like protease/PLproTM polyprotein produced by MERS-CoV and SARS-CoV induces the formation of autophagosomes, but inhibits their maturation, blocking the autophagosome-lysosome fusion and autophagic flux. Thus, these data suggest an antiviral role for autophagy.^{97,98} SARS-CoV papain-like protease (PLpro) and SARS-CoV-2 PLpro share 82.9% sequence identity.⁹⁹ PLpro functions also include the processing of the large polyproteins, pp1a and pp1ab produced by (ORF1a and ORF1ab), processing is essential for the release of 16 non-structural proteins (nsp1–16) of HCoV. The replicase complex formation essential for viral genome replication is dependent on nsp.^{100,101}

Shi et al. suggested that SARS-Coronavirus Open Reading Frame-8b (ORF8b) triggers intracellular stress pathways³². They

reported that ORF8b forms intracellular aggregates dependent on a valine at residue 77, those intracellular protein aggregates and misfolded proteins leads to ER stress and induction of the UPR, which contributes to the induction of lysosomal stress, autophagy, and eventual cell death. Furthermore, under ER stress, glucose-regulated protein 78 (GRP78) is overexpressed, which may result in its translocation to the cell surface. Ibrahim et al. proposed that the Spike protein has the capacity to invade the cell through molecules other than ACE2¹⁰². The S-protein of SARS-CoV-2 can bind to GRP78 on the cell surface, thus mediating virus entry into the cell. The mechanisms associated with autophagy triggered by coronaviruses is summarized in Figure 3.

Several potential drug candidates against COVID-19s chloroquine (CQ) and hydroxychloroquine (HCQ) are autophagy modulators.^{97,98,102–105} But interestingly those drugs seem to inhibit autophagy flux, similarly to the virus effects. Shojaei et al. suggest in a recent study that the effect against the disease of these drugs is “possibly due to the over-accumulation of autophagosomes that can potentially induce apoptotic cell death of virally infected cells and disrupt the virus replication cycle”⁹⁸. In addition to the anti-inflammatory effects by blocking the signaling of endosomal toll-like receptors, increasing the endolysosomal pH would also make it difficult to fuse the virus membrane with the endosomal membrane, preventing the virus from entering the cell. Unfortunately, clinical studies have shown that there is no improvement when these antimalarial drugs are given to hospitalized patients or in post-exposure prophylaxis¹⁰⁶.

The role of autophagy in coronavirus infection remains controversial. More work is needed to clarify its function in SARS-CoV-2 cell injury.

4 | CORONAVIRUS AND PROGRAMMED NECROTIC CELL DEATH

4.1 | Pyroptosis

Pyroptosis is a form of necrotic cell death that has been identified as caspase 1-dependent necrosis for a long time. Caspase 1 activation induces the maturation and secretion of proinflammatory cytokines, and consequently cell death occurs as result of gasdermin D-regulated (GSDMD-regulated) membranous pore formation in the plasma membrane. This process eventually leads to cell swelling and osmotic lysis with release of inflammatory intracellular contents. Caspase 1 is activated by several inflammasome, multiprotein complexes that assemble in response to host or infectious agent derived danger signals.^{107–109}

SARS-CoV Viroporin 3a activates the Nod-like receptor family, NOD-, LRR- and pyrin domain-containing protein 3(NLRP3) inflammasome by its ion channel (IC) activity. Based on data of previous study, it is known that 3a protein of SARS-CoV acts as a K⁺ channel and K⁺ efflux is a well-known activator of the NLRP3 inflammasome. Chen et al. suggested that SARS-CoV 3a protein disrupts intracellular ionic concentrations and causes mitochondrial damage, leading to NLRP3 inflammasome activation, which may result in pyroptosis (Figure 4a).²⁶ SARS 3a also has the ability to activate caspase-1 directly by its monomers or via K⁺ efflux by its oligomers, which results in the activation of NLRP3 inflammasome, contributing to pyroptotic cell death.²⁴

Nieto-Torres et al. showed that SARS-CoV envelope (E) gene encodes a small multifunctional protein that possesses IC activity, an important function in virus-host interaction.¹¹⁰ The results suggest

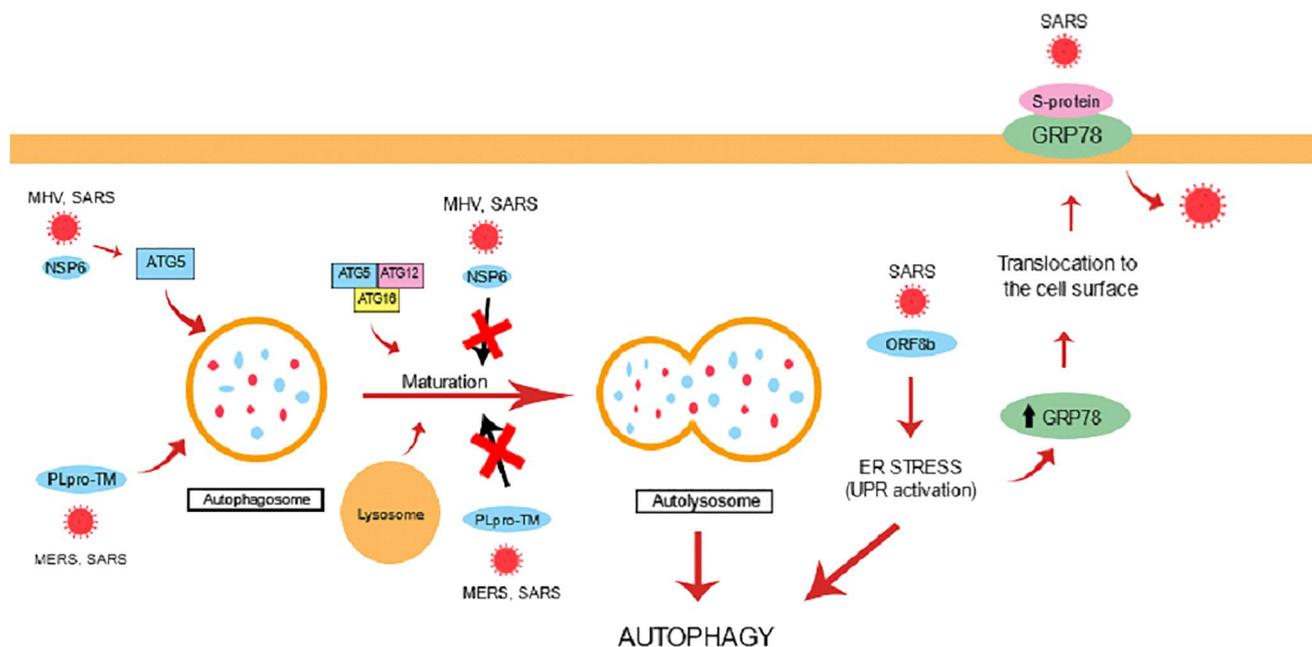


FIGURE 3 Mechanisms associated with autophagy triggered by coronaviruses

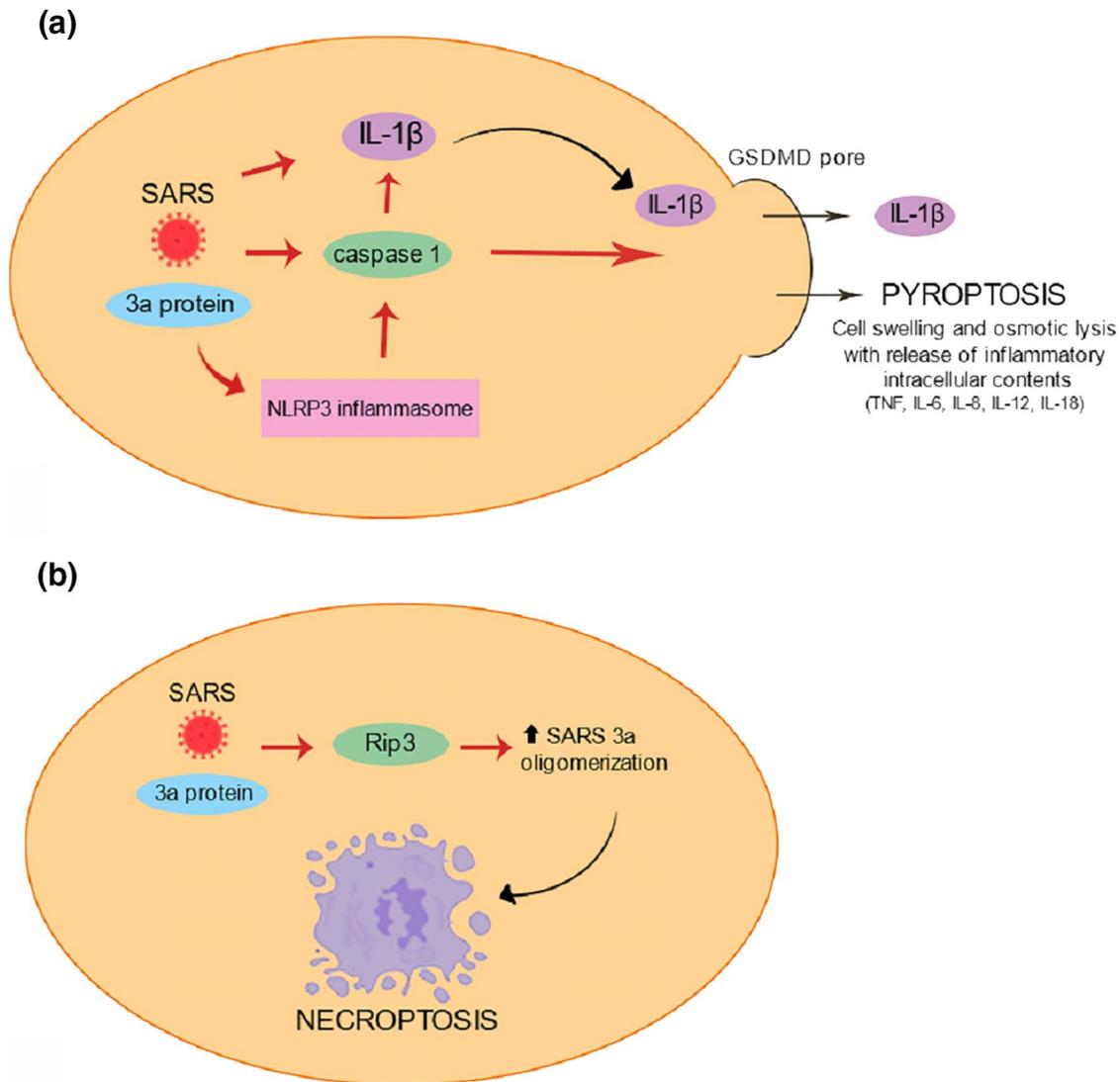


FIGURE 4 (a)–Pyroptosis triggered by the 3a protein of SARS-CoV; (b)–3a protein of SARS-CoV leads to necroptotic cell death by the ability of the SARS 3a protein to oligomerize and insert into membranes

that E protein IC activity induced edema accumulation and triggers inflammasome resulting in the production of IL-1 β , TNF and IL-6. Jiang et al. described that MERS-CoV infection also induced pyroptosis.¹¹¹ When infected with MERS-CoV, human DPP4 transgenic mouse model overexpressed caspase-1 in the spleen and showed high IL-1 β levels in serum. Inhibition of complement receptor C5aR1 reduced pyroptosis, suggesting that MERS-CoV infection induces overactivation of complement, which may contribute to pyroptosis and inflammation.

A clinical and pathological investigation of patients with severe COVID-19 performed by Li et al. showed that levels of TNF α and other factors were dramatically upregulated in blood, as well as severe inflammatory infiltrates in lung tissues of those patients.²⁵ The release of such molecules indicates that pyroptosis is the most frequent pattern of pulmonary injury. More recently, Rodrigues S et al. suggested that NLRP3 inflammasome is activated in response to SARS-CoV-2 infection and it is active during the disease.¹¹² Besides finding active NLRP3

inflammasome in PBMCs and tissues of post-mortem patients upon autopsy, the inflammasome-derived products such as Casp1p20 and IL-18 in the sera, correlated with the markers of COVID-19 severity, including IL-6 and lactate dehydrogenase. Ferreira et al. also showed that SARS-CoV-2 engages inflammasome and pyroptosis in human primary monocytes.¹¹³ According to their findings, SARS-CoV-2 induced pro-caspase-1 cleavage, which is known to trigger IL-1 β production and GSDMD cleavage, leading to pyroptosis.

4.2 | Necroptosis

Necroptosis is a caspase-independent form of programmed necrotic cell death, and its pathway is triggered by several stimuli, including death receptor ligands. Using standard histological techniques, morphologically there are no differences between cells that are undergoing necrosis and necroptosis. While apoptosis and other forms

of programmed cell death have caspases as key role, necroptosis is regulated by receptor-interacting protein kinases (RIPK). The cell death mechanism is activated by RIPK1 and requires RIPK3-dependent phosphorylation of mixed-lineage kinase domain-like pseudokinase (MLKL), resulting in MLKL oligomerization. It induces plasma membrane rupture and consequently cell death with release of damage-associated molecular patterns.^{114–116}

Yue et al. described that SARS-Coronavirus open Reading Frame-3a (SARS 3a) drives multimodal necrotic cell death. The study showed that SARS 3a protein interacts with receptor interacting protein 3 (Rip3), which augments the oligomerization of SARS 3a protein. This mechanism probably leads to necroptotic cell death by the ability of the SARS 3a protein to oligomerize and insert into membranes as phosphorylated MLKL (Figure 4b). Furthermore, SARS 3a causes lysosome damage and dysfunction by inserting into lysosomal membranes, which leads to activation of transcription factor EB, increasing the transcription of autophagy- and lysosome-related genes.²⁴

The studies that focus on the cell death caused by Coronaviruses used in this review is summarized in Table 1.

5 | IN SILICO ANALYSIS TO IDENTIFY CELL DEATH PATHWAYS INVOLVED IN SARS-CoV INJURY

To identify the key cell death pathways related with SARS-CoV infection, we used text mining, using pubmed. mineR^{117,118} from PubMed abstracts. We performed the search using the same terms cited above and found 105 articles. Gene ontology term enrichment analysis of 19 genes and biological process were performed, and results were plotted in Figure 5.

Some of these genes such as SIRT4, TYRO3, GAS6, AXL, MERTK, EGFR and CCL2 are negative regulators of apoptosis, while the TLR4, SIRT2, CTLA4 and HMGB1 genes are positive regulators. In contrast, the TNF and SIRT1 genes are concomitantly positive and negative regulators of apoptosis. In addition, the TNF, TLR4 and IRF3 genes appear to be involved in the regulation of necrosis and apoptosis. The specific functions of each gene in the figure above are described in Table S2 of the Supplementary Material.

TABLE 1 Cell death and CoVs

Autor	Ano	CoV	CellLine	Tissue	Cell death	Reference
Surjit, Milan et al.	2004	SARS-CoV	COS-1, HuH7	Kidney	Apoptosis	29
Tan, Yee-Joo et al.	2004	SARS-CoV	HEK293T, HeLa, A549, HepG2, Vero E6, COS-7	Kidney, cervix, lung, liver,	Apoptosis	23
Ren, Lili et al.	2005	SARS-CoV	Vero	Kidney	Apoptosis	70
Law, Patrick T W et al.	2005	SARS-CoV	Vero E6	Kidney	Apoptosis	71
Kopecky-Bromberg, Sarah A et al.	2006	SARS-CoV	HEK293T, A549, and HeLa	Human embryonic kidney, lungs, cervix	Apoptosis	60
Khan, Sehaam et al.	2006	SARS-CoV	Vero E6	Kidney	Apoptosis and necrosis	52
Chan, Chak-Ming et al.	2007	SARS-CoV	HEK293T, TransgenicDrosophila	Kidney	Apoptosis	24
Chen, Chia-Yen et al.	2007	SARS-CoV	VeroE6, HEK 293T, and HuH-7	Kidney human embryonic kidney	Apoptosis	52
Zhang, Lu et al.	2007	SARS-CoV	COS-1	Kidney	Apoptosis	75
Tan, Ying-Xim et al.	2007	SARS-CoV	VeroE6, HEK293T	Kidney	Apoptosis	62
Chan, E et al.	2008	SARS-CoV	Vero E6 e 3a TransgenicDrosophilamodel	Kidney	Apoptosis	31
Padhan, Kartika et al.	2008	SARS-CoV	Huh7	Kidney	Apoptosis	22
Ye, Zhongde et al.	2008	SARS-CoV	Vero E6, COS-7 cells and HEK293T cells	Kidney and human embryonic kidney	Apoptosis	58
Minakshi, Rinki et al.	2009	SARS-CoV	COS-1, Vero, Huh7	Kidney	Apoptosis	28
Freundt, Eric C et al.	2009	SARS-CoV	Vero cells	Kidney	Apoptosis	73
Ye et al.	2010	SARS-CoV	Vero E6 and COS-7 cells	Kidney	Apoptosis	57
Sharma, Kulbhushan et al.	2011	SARS-CoV	Vero cells	Kidney	Apoptosis	67
Tsoi, Ho et al.	2014	SARS-CoV	HEK293FT	Kidney	Apoptosis	27
Nieto-Torres, Jose L et al.	2014	SARS-CoV	Vero E6, BHK-21 cells	Kidney	Pyroptosis	110

TABLE 1 (Continued)

Autor	Ano	CoV	CellLine	Tissue	Cell death	Reference
Chu, Hin et al.	2016	MERS-CoV	Peripheral blood mononuclear cells, Tcells and tonsil and spleen-dissociated cells	Blood	Apoptosis	76
Yeung, Man-lung et al.	2016	MERS-CoV	Calu-3 and normal human mesangial cells	Lungs, kidney	Apoptosis	79
Yue, Yuan et al.	2018	SARS-CoV	HeLa, HEK 293T, A549, Thp-1	Cervix, kidney, lung, peripheral blood	Necrosis	24
Chen, I-Yin et al.	2019	SARS-CoV	BMMs, HEK293FT, HeLa, HT-1080	Kidney, Cervix, connective tissue	Pyroptosis	26
Shi, Chong-Shan et al.	2019	SARS-CoV	THP-1, HEK293, A549, HeLa	Peripheral blood, kidney, lung, cervix	Autophagy	32
Jiang, Yuting et al.	2019	MERS-CoV	Human monocytic cells (THP-1)	Blood	Pyroptosis	111
Carmona-Gutierrez, Didac et al.	2020	MERS-CoV, SARS-CoV	-	-	Autophagy	54
Li, Shaohua et al.	2020	SARS-CoV-2	-	-	Pyroptosis	25
Li, Shufen et al.	2020	SARS-CoV-2	Calu-3, Vero, Vero E6, THP-1	Lung	Apoptosis Necroptosis	91
Shojaei, Shahla et al.	2020	SARS-CoV-2	-	-	Autophagy	53
Bellesi, Silvia et al.	2020	SARS-CoV-2	Peripheral blood T Lymphocytes	Blood	Apoptosis	77
Ren, Yujie et al.	2020	SARS-CoV-2	VeroE6, HEK293T and HepG2	Kidney, lung	Apoptosis	72
Rodrigues, Tamara S et al.	2021	SARS-CoV-2	Peripheral blood mononuclear cells	Blood	Pyroptosis	112
Ferreira, André C et al.	2021	SARS-CoV-2	Vero E6, human primary monocytes	Kidney, blood	Pyroptosis	113

Abbreviations: CoVs, coronaviruses; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus.

5.1 | Enrichment analysis for SARS-Human proteins interactions

To better understand what interactions the SARS proteins could potentially have with the human host and its death-processes associated proteins, we performed another *in silico* analysis. We used interaction data from the p-HIPSTER tool, which is an algorithm for predicting interactions between viral and human proteins based on similar interactions of reference viral proteins and the human protein. Viral protein data from p-HIPSTER corresponds to SARS-CoV, not yet to SARS-CoV 2, and because of this our results should have preliminary and exploratory purposes only.

For each viral protein a list of targets was predicted by P-HIPSTER as possible interactors. With these list of targets, an enrichment analysis for Gene Ontology Biological Processes was performed using g:Profiler and a heat map was made to show the negative log₁₀ of adjusted *p*-value for enrichment of death-related biological process in these interactors lists.

According to p-HIPSTER results, the ORF7b had only one interaction (PCNA protein) and no interactors resulted from M

protein prediction on p-HIPSTER. For both of these two, no enrichment analysis could be performed on g:Profiler due to not enough predicted interacting proteins.

As presented in Figure 6, many SARS-CoV proteins (N, NSP1, NSP10, NSP4, NSP5, NSP7, ORF7a and ORF9b) show interactions with human proteins strongly associated with death biological processes. Some others (NSP12, NSP13, NSP9, NSP8 and Spike) were just slightly associated to death biological processes. However, many other SARS proteins are not associated with any death biological processes (E, NSP14, NSP15 and NSP16). Although NSP14 shows no death-related biological processes enriched among its predicted interactors, it was due to only few human targets predicted as interactors (ISG15 and UBC).

All SARS proteins which had their targets enriched with apoptotic functions were more associated with its positive regulation than negative. Although not closely related to it, the nucleocapsid protein and NSP3 had a mild enrichment with human protein interactors functionally associated with autophagy biological processes. Also, according to our analysis necrosis-related biological processes were enriched for none of the viral protein interactors lists.

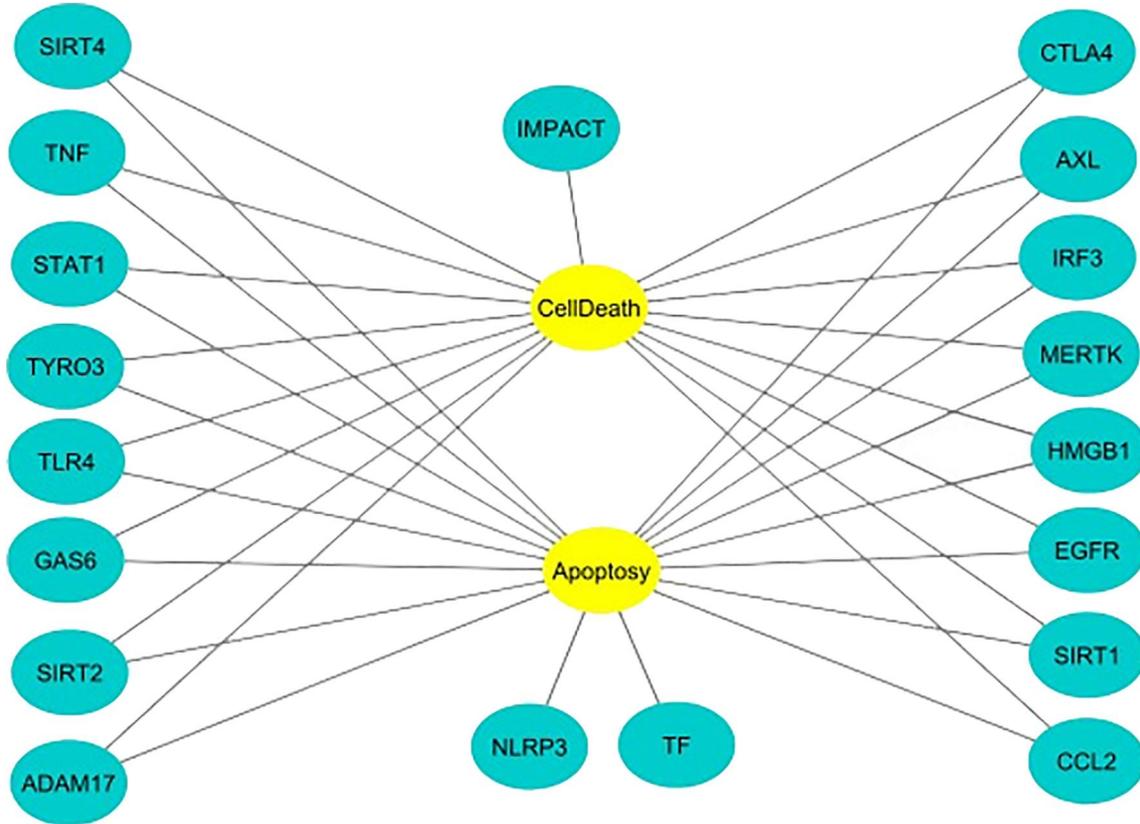


FIGURE 5 Biomolecular interaction network with genes found by text-mining analysis

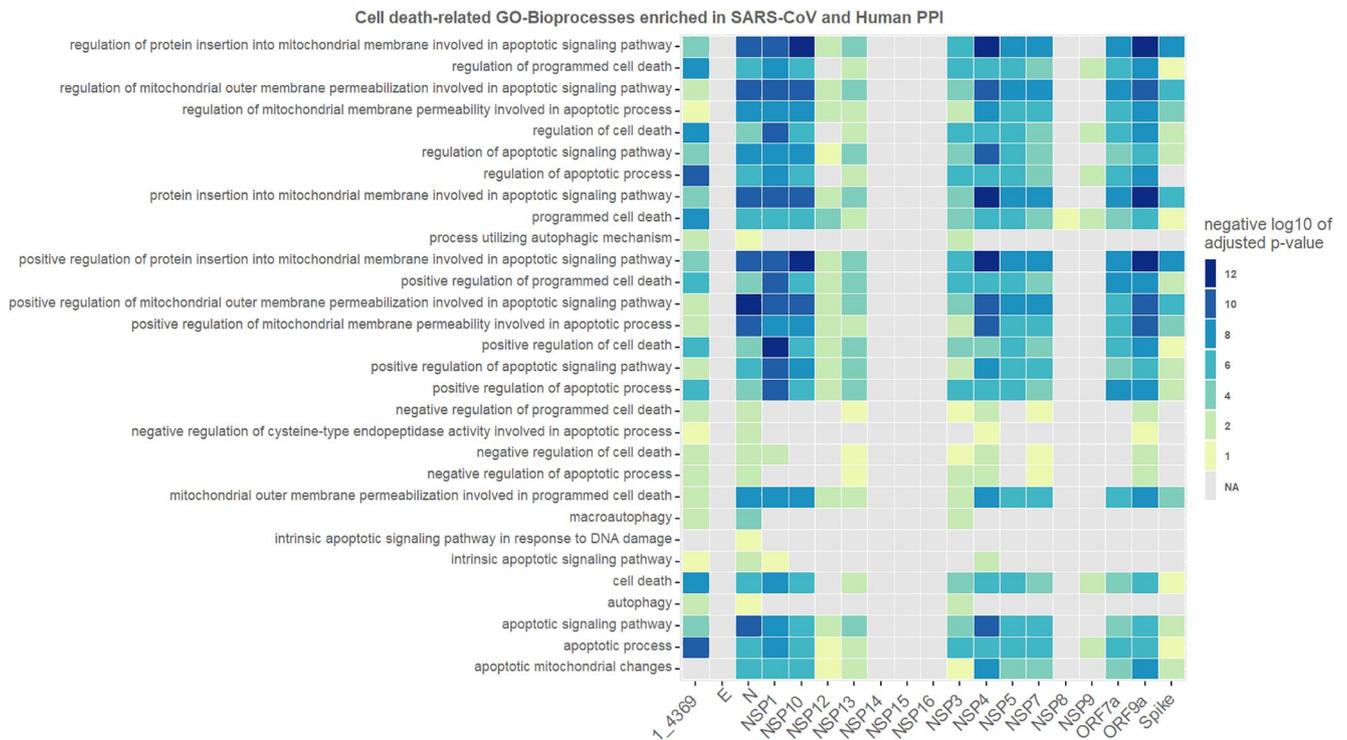


FIGURE 6 Heat map enrichment of death-related biological process and genes of Sars-CoV (negative log10 of adjusted p-value)

Many of the proteins identified in the *in silico* analyses are not the same as those addressed in the first section of the study, indicating that this topic still needs to be explored further.

6 | CONCLUSIONS

Coronaviruses can induce different forms of cell death, triggered by several signaling pathways. Understanding the molecular mechanisms of cell death that cause tissue and cell injuries by SARS-CoV-2 may contribute to finding potential therapeutic targets against the current pandemic. Functional studies with recombinant viruses with specific deletions or mutations, or with transfection vectors containing SARS proteins will be important to determine the relative contribution of these proteins to the cell death mechanism of SARS-CoV-2 injuries.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Marillya Morais da Silva, Vanessa Mylenna Florêncio De Carvalho, Priscilla Stela Santana de Oliveira -writing-original draft and editing. André Silva Lira de Lucena, Sergio de Sá Leitão Paiva Júnior - *in silico* analysis and writing; Michelle Melgarejo da Rosa, Moacyr Jesus Barreto de Melo Rego, Maira Galdino da Rocha Pitta - reviewing, and editing; Michelly Cristiny Pereira - conceptualization, preparation, writing, reviewing, and editing the final manuscript.

DATA AVAILABILITY STATEMENT

All data used are available in this review.

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