



HAL
open science

Digesting the crisis: autophagy and coronaviruses

Didac Carmona-Gutierrez, Maria A Bauer, Andreas Zimmermann, Katharina Kainz, Sebastian J Hofer, Guido Kroemer, Frank Madeo

► **To cite this version:**

Didac Carmona-Gutierrez, Maria A Bauer, Andreas Zimmermann, Katharina Kainz, Sebastian J Hofer, et al.. Digesting the crisis: autophagy and coronaviruses. *Microbial Cell* , 2020, 7 (5), pp.119-128. 10.15698/mic2020.05.715 . inserm-02612304

HAL Id: inserm-02612304

<https://inserm.hal.science/inserm-02612304>

Submitted on 19 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Digesting the crisis: autophagy and coronaviruses

Didac Carmona-Gutierrez^{1,*}, Maria A. Bauer¹, Andreas Zimmermann^{1,2}, Katharina Kainz¹, Sebastian J. Hofer¹, Guido Kroemer³⁻⁷ and Frank Madeo^{1,2,8,*}

¹ Institute for Molecular Biosciences, NAWI Graz, University of Graz, Graz, Austria.

² BioHealth Graz, Graz, Austria.

³ Centre de Recherche des Cordeliers, Equipe labellisée par la Ligue contre le cancer, Université de Paris, Sorbonne Université, INSERM U1138, Institut Universitaire de France, Paris, France.

⁴ Metabolomics and Cell Biology Platforms, Institut Gustave Roussy, Villejuif, France.

⁵ Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France.

⁶ Suzhou Institute for Systems Medicine, Chinese Academy of Medical Sciences, Suzhou, China.

⁷ Karolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden.

⁸ BioTechMed Graz, Graz, Austria.

* Corresponding Authors:

Didac Carmona-Gutierrez, Institute of Molecular Biosciences, Humboldtstrasse 50/EG, 8010 - Graz, Austria;

E-mail: carmonag@uni-graz.at;

Frank Madeo, Institute of Molecular Biosciences, Humboldtstrasse 50/EG, 8010 - Graz, Austria; E-mail: frank.madeo@uni-graz.at

ABSTRACT Autophagy is a catabolic pathway with multifaceted roles in cellular homeostasis. This process is also involved in the antiviral response at multiple levels, including the direct elimination of intruding viruses (virophagy), the presentation of viral antigens, the fitness of immune cells, and the inhibition of excessive inflammatory reactions. In line with its central role in immunity, viruses have evolved mechanisms to interfere with or to evade the autophagic process, and in some cases, even to harness autophagy or constituents of the autophagic machinery for their replication. Given the devastating consequences of the current COVID-19 pandemic, the question arises whether manipulating autophagy might be an expedient approach to fight the novel coronavirus SARS-CoV-2. In this piece, we provide a short overview of the evidence linking autophagy to coronaviruses and discuss whether such links may provide actionable targets for therapeutic interventions.

doi: 10.15698/mic2020.05.715

Received originally: 15.04.2020;

in revised form: 30.04.2020,

Accepted 01.05.2020.

Published 04.05.2020.

Keywords: COVID-19, SARS, SARS-CoV-2, MERS, coronavirus, virophagy, inflammation, immunity.

Abbreviations:

CoV – coronavirus; COVID-19 – Coronavirus Disease 2019; DMV – double membrane vesicle; hCoV – human pathogenic CoV; HIV – human immunodeficiency virus; HSV – herpes simplex virus; LIR – LC3-interacting region; MERS – Middle East respiratory syndrome; MHC – major histocompatibility complex; MHV – mouse hepatitis virus; PAMP – pathogen-associated molecular pattern; PEDV – porcine epidemic diarrhea virus; PRR – pattern recognition receptor; SARS – severe acute respiratory syndrome; TGEV – transmissible gastroenteritis virus; UBD – ubiquitin-binding domain.

INTRODUCTION

The ongoing outbreak of the respiratory disease COVID-19 (Coronavirus Disease 2019) is endangering individuals, governments and societies around the world. Indeed, this pandemic has unprecedented global consequences at the economic and social levels as it challenges the medical and research communities. The first case of COVID-19 was reported in December 2019 in Wuhan (China) [1] and, despite increasingly drastic efforts to contain the disease, the

infection rapidly spread across the planet, leading the WHO to declare it, first, a global health emergency (in January 2020) and eventually a pandemic (on March 11, 2020) [2, 3]. As of April 30th 2020, more than 200 countries and territories have reported COVID-19 cases with a total of over three million confirmed cases and more than 220.000 confirmed deaths [4]. In the current absence of effective treatments and vaccines, these numbers will continue to grow inexorably. The main clinical features of COVID-19

(fever, dry cough, pneumonia) manifest after an average incubation period of approximately 5 days [5, 6] with severe cases advancing towards severe acute respiratory syndrome (SARS) [7–9]. COVID-19 was quickly shown to be caused by a novel coronavirus (CoV) that was named SARS-CoV-2 [7, 10].

SARS-CoV-2 is structurally related to the members of the *Coronaviridae* family in the order *Nidovirales* [10, 11]. CoVs are enveloped viruses with a single-strand, positive-sense RNA genome and are separated into four genera according to their genome characteristics: α -CoV, β -CoV, γ -CoV, and δ -CoV [12, 13]. Until the emergence of COVID-19, six human pathogenic CoVs (hCoVs) had been identified: four non-SARS-like hCoVs (HCoV 229E, NL63, OC43, and HKU1), which do not cause major (fatal) epidemics, and two SARS-like hCoVs (SARS-CoV and Middle East respiratory syndrome coronavirus MERS-CoV) [14]. Both SARS-like hCoVs, which belong to the β -CoV genus, have already caused major outbreaks and health emergencies in China (SARS-CoV, 2002-2003) as well as in the Middle East (MERS-CoV, 2012) [15]. The novel SARS-CoV-2 increases the number of known hCoVs to seven, all of which have a zoonotic origin, meaning that they originate from vertebrate animals (possibly bats for SARS-CoV-2) and have reached humans via interspecies transmission [16].

AUTOPHAGY AS A PROTECTIVE CATABOLIC PROCESS

Given the devastating scenario that COVID-19 has already generated, a multitude of potential approaches against SARS-CoV-2 are currently being conceived. In that sense, we think that the manipulation of autophagy might be worth exploring. Macroautophagy (hereafter referring to autophagy) is an intracellular catabolic mechanism that regulates the elimination of unwanted, superfluous or defective cell components [17]. In this process, the targeted molecules/organelles are enclosed in double-membrane vesicles called autophagosomes, which eventually fuse with lysosomes for degradation of the delivered contents. This process is induced upon energy deprivation and/or physical activity in order to secure sufficient nutrient supply and is thus often referred to as a cellular self-digestion system. The compounds resulting from lysosomal degradation (e.g., fatty acids, amino acids) may be released into the cytoplasm for the generation of new macromolecules and/or bioenergetics reactions. Autophagy, therefore, represents a recycling mechanism under circumstances of low resource availability [17].

The digestion of damaged and thus potentially detrimental cargo also increases the fitness at the cellular and consequently at the organismal level. Accordingly, the induction of autophagy by dietary interventions (e.g., caloric restriction, fasting regimens), behavioral cues (e.g., exercise) or pharmacological agents (e.g., caloric restriction mimetics like spermidine, rapamycin, resveratrol or specific chalcones [18–22]) has been consistently linked to beneficial effects on health [23]. In turn, reduced autophagic capacity is connected to aging progression and a number of

pathologies including cardiovascular disease, cancer and neurodegeneration [24].

The autophagic machinery can retrieve its cargo in bulk, but it can also selectively target distinct intracellular components and organelles via specific adapter proteins that interact with both the individual substrates and the autophagosomes [25]. Specifically, these autophagy adaptors (e.g., p62/SQSTM1, optineurin) contain both a ubiquitin-binding domain (UBD) and an LC3-interacting region (LIR). The UBD recognizes ubiquitin tags decorating the specific target, while the LIR allows binding of LC3 proteins attached to nascent phagophores, the structures that eventually close to form autophagosomes [26]. Accordingly, autophagic subforms have been defined that describe, for example, the specific removal of damaged mitochondria (mitophagy), the disposal of protein aggregates (aggrephagy), the digestion of lipid droplets (lipophagy), etc [27].

Yet another selective autophagy subroutine is xenophagy, the specialized elimination of intracellular pathogens, including fungi, bacteria and viruses [28]. The xenophagic disposal of viruses (sometimes also termed virophagy) has been described for different viral pathogens, including human immunodeficiency virus-1 (HIV-1) [29, 30] and herpes simplex virus-1 (HSV-1) [31, 32]. However, many viruses (including HIV-1 and HSV-1) and other pathogens have also evolved strategies to escape or inhibit autophagy and sometimes even to manipulate the autophagic machinery for their replicative benefit [33]. Thus, autophagy emerges as a crucial element in the evolutionary-driven arms race of host against pathogen. This further underlines the significance of xenophagy as a central antimicrobial mechanism, but also limits the potential of autophagy induction to fight infections.

CORONAVIRUSES AND AUTOPHAGY

The question whether autophagy induction might be beneficial to specifically counteract SARS-CoV-2 infection cannot be answered at this point. However, the existing data on other CoVs suggest that – despite some conflicting results – autophagy induction might be a valid approach that should be subjected to further evaluation. The autophagy-lysosomal system does indeed seem to play a central role during the infection with different CoVs, including SARS-CoVs [34–37]. Nevertheless, the diverse putative roles of autophagy during viral infection [38] require that two opposite aspects be covered by this overview: (i) Is there evidence for autophagy being used for the survival or replication of CoVs (proviral effects)? (ii) Can autophagy induction reduce cellular infection, and does the virus actively inhibit autophagy (suggesting antiviral effects for autophagy)? Of note, cellular manipulation of autophagic levels during infection may also reflect desperate attempts of the cell to reestablish homeostasis, either through restriction of viral entry by actively shunting endocytosis/endosomal trafficking (possibly resulting in autophagy reduction as a side-effect) [39] or to counteract virally induced cell death by increasing cytoprotective autophagy. As a third major as-

pect, (iii) the autophagic effects on antiviral immunity must be taken into consideration. Over the last 15 years, a number of studies have addressed these issues in the context of the “old” CoVs, those that historically precede SARS-CoV-2.

Can autophagy be hijacked for CoV replication?

CoVs rely on the formation of replication complexes at double membrane vesicles (DMVs), where viral genome replication and transcription occurs [40, 41]. As it may be at least partly the case for autophagosomes, DMVs probably depend on ER-derived membranes for their generation [40, 42]. Therefore, it was initially suspected that CoVs might usurp the autophagosomal machinery for DMV formation. In fact, an early study using *ATG5* knockout cells implied that autophagy may promote mouse hepatitis virus (MHV) replication via formation of DMVs [43]. In a follow-up work, the same authors reported the co-localization of specific replication proteins (nsp2, nsp3, nsp8) with endogenous LC3, suggesting a connection between replication and autophagosome formation [44]. Other studies reported that the porcine CoVs transmissible gastroenteritis virus (TGEV) [45] and porcine epidemic diarrhea virus (PEDV) [46] may induce autophagy to promote replication. However, the link between autophagy and replication of CoVs has been challenged by a number of different reports, showing that deletion of the essential autophagy genes *ATG5* or *ATG7* does not affect the replication of MHV [47, 48] or SARS-CoV [49]. Furthermore, the co-localization of LC3 (or GFP-LC3) with the SARS-CoV RNA replication complex could not be confirmed [50]. A study using pharmacological induction (rapamycin) or genetic inhibition (knock-down of LC3, *ATG5*, or *ATG7*) of autophagy even demonstrated that TGEV replication is negatively regulated by autophagy [51]. In addition, rapamycin reduced infectivity of PEDV [52]. Altogether, although some conflicting results exist, autophagy may not be primarily engaged in promoting CoV replication.

However, single components of the autophagic machinery may be hijacked independently of their activity in autophagic processing. Non-lipidated LC3, for instance, is used for membrane derivation for DMVs in CoVs, and downregulation of LC3 but not inactivation of autophagy, counteracts CoV infection [47]. Notably, some components of the canonical autophagy machinery can target invading pathogens by promoting phagosome-to-lysosome fusion [53]. During LC3-associated phagocytosis (LAP), for instance, which does not involve sequestration in autophagosomes, LC3 is recruited to phagosome membranes to facilitate lysosomal fusion and degradation [54]. In addition, it has been proposed that non-canonical autophagy and intruding viruses, both of which can be inhibited by the fungal compound brefeldin A, may converge in the retrieval of membranes [55]. Non-canonical autophagy includes *ATG5*- and *ATG7*-independent as well as Beclin-1-independent autophagy [56, 57]. Altogether, these observations add further layers of possible modulation of autophagic processes in trying to attenuate viral infection (Figure 1).

Does autophagy counteract CoV infection?

Intriguingly, a body of evidence indicates that CoVs may actually inhibit autophagy, which in turn teleologically suggests an antiviral role for autophagy. Accordingly, a number of studies have shown that increasing autophagic capacity may be beneficial against infection (Figure 1). A temporal kinome analysis of MERS-CoV-infected hepatocytes demonstrated selective activation of the ERK/MAPK and PI3K/AKT/mTOR signaling responses, both of which have inhibitory effects on autophagy [58]. Accordingly, pharmacological inhibition of these pathways was able to inhibit MERS-CoV infection [58]. Moreover, although one of the central replicase proteins (nsp6) of avian infectious bronchitis virus (IBV) seems to promote autophagosome formation at the omegasome level [59], nsp6 also limits further autophagosomal expansion [60], thus compromising autophagic delivery of viral components to lysosomes. Interestingly, nsp6 is also present in other CoVs, including SARS-CoV-2 [61].

Overexpression of the protease PLP2 of SARS-CoV and MERS-CoV in different cell lines inhibited autophagosome-lysosome fusion and autophagic flux [62]. A recent report corroborated the autophagy-inhibitory activity of MERS-CoV and showed that induction of autophagy can reduce MERS-CoV replication [37]. Specifically, the authors found that pharmacological inhibition of the ubiquitin ligase SKP2 increases the levels of Beclin-1, a central regulator of phagophore formation [37]. SKP2 executes lysine-48-linked poly-ubiquitination of Beclin-1, targeting it for proteasomal degradation, the inhibition of which promoted autophagy and efficiently reduced MERS-CoV replication. This work also shows that ectopic expression of at least three viral proteins (nsp6, p4b, p5) restricts autophagy [37]. While nsp6 may limit autophagosome expansion (see above), p4b inhibits RNase L activation (a pro-autophagic event [63]), and p5 has been connected to the blockage of IFN- β induction [63, 64], which itself may be linked to autophagy [65]. Accordingly, deletion of p4b or p5 resulted in reduced MERS-CoV growth, although disputing some previous observations, which the authors explain by methodological differences [37]. Thus, the group-specific accessory proteins, which by definition are not essential for viral replication but are involved in the modulation of host cells and immune evasion [66, 67], may represent targets for reducing the autophagy-inhibitory effects of CoVs.

The FDA-approved anti-malarial drugs chloroquine and hydroxychloroquine have been suggested to be repurposed for the treatment of COVID-19 [68–70], but this remains widely controversial [71–73]. Although chloroquine is a lysosomotropic agent that blocks autophagic degradation, possibly by impairing autophagosome fusion with lysosomes [74], the putative effects on autophagy may not be necessarily causal for the antiviral activity. In fact, endosomal acidification after endocytosis is critical for SARS-CoV-2 entry [75], and chloroquine inhibits this acidification [76]. In addition, chloroquine limits terminal glycosylation of the metalloproteinase ACE2, the functional receptor for SARS-CoV and SARS-CoV-2 cell entry [68, 75, 77]. Non-glycosylated ACE2 seems to interact less efficiently with

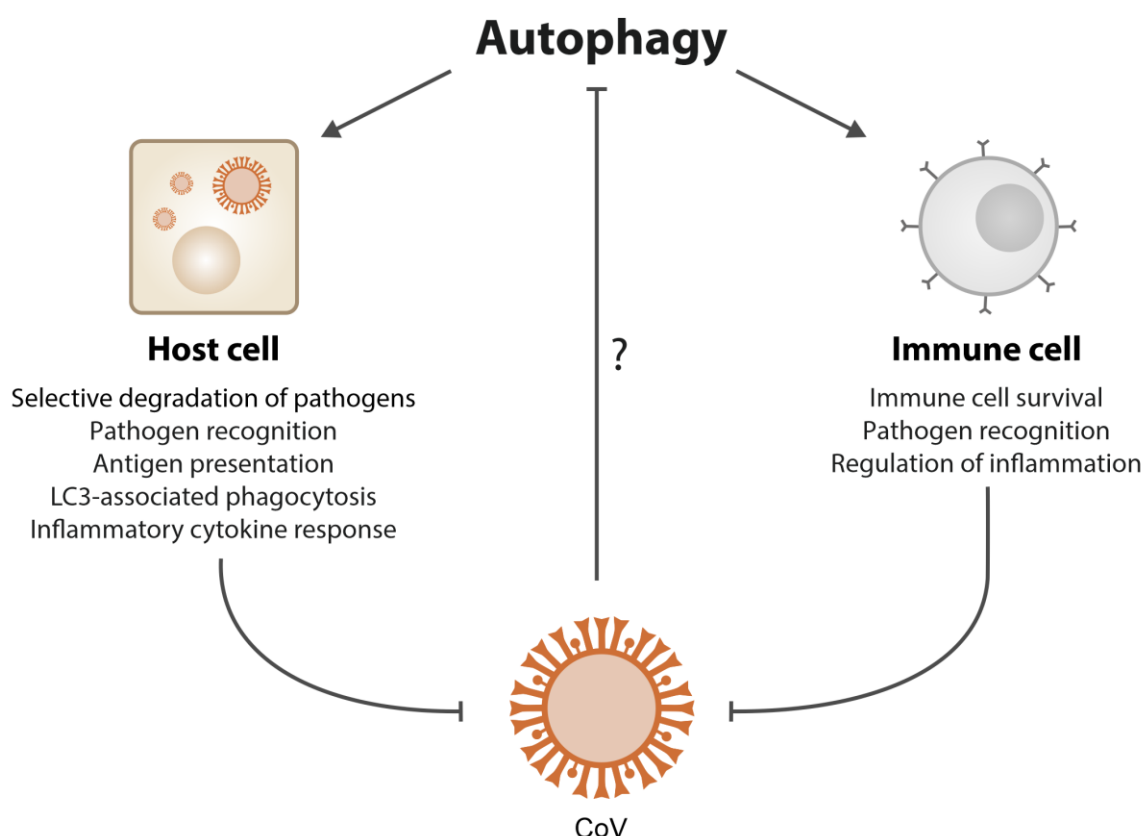


FIGURE 1: Possible protective mechanisms of autophagy against coronaviruses. Autophagy may counteract viral infection by executing and supporting different antiviral pathways in infected host cells as well as by promoting the function and survival of immune cells. Therefore, an increase in autophagic flux may be effective against coronaviruses (CoVs). Accordingly, CoVs may have developed strategies to limit cellular and organismal immunity by preventing autophagy.

the SARS-CoV spike protein, thus reducing viral entry [78]. These modes of action would target the virus upstream of autophagy, making it unlikely that autophagy modulation contributes to the outcome of chloroquine treatment at that point. Additionally, chloroquine has been shown to induce autophagy-independent effects, for instance, Golgi disorganization [74] and pulmonary vasodilation [79] that may contribute to its controversial clinical activity. Interestingly, the net effects on autophagy may differ depending on parameters like cell type and dose: for instance, chloroquine-mediated lysosomal stress may promote the nuclear translocation of the pro-autophagic transcription factors TFEB and TFE3 [80, 81]. Altogether, further clinical work must clarify if chloroquine and hydroxychloroquine have major effects on COVID-19 and at which (early?) phase of the disease these drugs should be administered. If chloroquine or its derivative turned out to be clinically useful against COVID-19, it will be important to understand to which extent such effects are connected to the modulation of autophagy.

Indeed, repurposing of known (especially currently FDA-approved) drugs might offer a welcome shortcut to rapidly developing treatments against COVID-19. Obviously, even in the present period of desperate search for pharmacological treatments, the efficacy (and lack of side-effects) of anti-COVID-19 treatments needs to be proven by clinical studies subjected to rigorous scientific scrutiny. While data regarding such attempts remain preliminary at this stage, several currently available preprints allow the speculation of autophagy as a possible druggable target. For instance, a recent preprint mapping the SARS-CoV-2 interactome in human host cells identifies several host factors connected to autophagy [82]. Amongst others, the authors find viral-human interactions with proteins directly modulated by mammalian target of rapamycin complex 1 (mTORC1) [82], a master regulator of cell proliferation and autophagy known to be affected by other viruses [83, 84].

Intriguingly, another recent preprint presents *in vitro* data showing that SARS-CoV-2 infection restricts autophagy and that, in turn, pro-autophagic compounds - including spermidine - may inhibit viral propagation [85]. Admittedly,

spermidine has a plethora of physiological functions, including regulation of RNA-to-protein translation, as well as amelioration of RNA and DNA stability [86], and these effects need further investigation, specifically in relation with a possible autophagy induction. In fact, the role of spermidine during infection may be pleiotropic. On the one hand, spermidine is an autophagy inducer [18, 87–90] and may promote viral xenophagy; also, it may contribute to endosomal pH buffering [91] and thus block viral entry. On the other hand, polyamines are essential for viral replication [92], and the overexpression of spermidine/spermine-N(1)-acetyltransferase by the host, which drives spermidine (and spermine) depletion, is a common cellular response to viral infections, including that of RNA viruses [93]. This underlines once more that any pharmacological candidate for the fight against SARS-CoV-2 should be examined for its (possible) pleiotropy during infection.

The role of autophagy on immune signaling

Autophagy directly impacts immune signaling, and autophagy induction hence may counteract CoVs by promoting immunological defense mechanisms (Figure 1). For instance, autophagy regulates, and can be regulated by, so-called pattern recognition receptors (PRRs), which sense molecules specifically derived from microorganisms (pathogen-associated molecular patterns, PAMPs) [94, 95]. PRRs are present in various cells of the innate immune system and many epithelial cells [95]. There, they are distributed at diverse locations (either at the cell surface or in the cytoplasm), providing a recognition system for different life cycle phases of a given pathogen [95]. PAMP-induced signaling triggers a multitude of pathways, including pro-inflammatory responses, that result in the synthesis of immunostimulatory molecules including cytokines, chemokines and immunoreceptors [94].

Autophagy further assists immune responses by processing and supporting the presentation of antigens on major histocompatibility complex (MHC) class II, responsible for loading extracellular antigens [96, 97]. In addition, autophagy can deliver exogenous viral antigens into MHC-I molecules for cross-presentation (the MHC-I pathway is usually employed for loading endogenous antigens) [97, 98]. Moreover, autophagy directly impacts the activation, proliferation and differentiation of immune cells [99]. Amongst others, autophagy has been connected to the differentiation of CD8⁺ T-cells into cytotoxic T lymphocytes, to dendritic cell and B cell development, to plasma cell differentiation, and to macrophage differentiation, therefore covering multiple instances of the innate and adaptive immune systems [99]. Of note, autophagy activation by caloric restriction mimetics (including hydroxycitric acid and spermidine) also improves anticancer immunosurveillance [100]. In addition, the general cellular pro-survival effects of autophagy extend to several immune cell types. Pharmacological autophagy induction by spermidine [87], for instance, has been demonstrated to promote the maintenance and survival of memory CD8⁺ T cells [101] as well as to counteract B lymphocyte senescence [102].

The interplay of autophagy and inflammation is complex, since it does not only encompass positive but also negative regulatory mechanisms. For example, autophagy can digest the interleukin precursors produced by inflammasomes (e.g. pro-IL-1), but also directly target inflammasome components (e.g. NLRP3, AIM2 and ASC) [103]. In sum, autophagy ensures acute inflammatory responses while preventing excessive and prolonged hyperinflammation.

Finally, it should be noted that autophagy may have paracrine functions in the form of unconventional secretion [104], thus adding yet another layer of non-cell autonomous effects. In the context of viral infections, this mechanism may be used to induce protective responses in cells neighboring an infection site [105, 106]. In primary human placental trophoblasts, for instance, autophagy induction promotes packaging and exosome-dependent delivery of specific miRNAs, which induce autophagy in non-placental recipient cells, conferring resistance to a variety of viruses [105]. This may be (one of) the mechanism(s) through which placental and maternal cells optimize their defense against viral infections during pregnancy.

CONCLUSIONS

The existing evidence allows the speculation that autophagy induction might counteract CoV infection at different levels, although more specific data are certainly required. As mentioned above, the restriction of calorie intake is the most robust method to induce autophagy. However, the fight against acute infections also requires sufficient energy supply, suggesting that autophagy induction via caloric restriction or fasting regimens may be counterproductive, at least in the short-term during (as well as shortly before) infection. Therefore, caloric restriction mimetics [107, 108], natural or synthetic compounds with the ability to induce autophagy, may circumvent this problem.

Irrespective of whether autophagy modulation will eventually be part of the strategies against COVID-19, the current pandemic outbreak is a shocking reminder that emerging (and re-emerging) infectious pathogens are (and will be) a major challenge. In view of the exposed vulnerability of our medical structures and socioeconomic well-being, this pandemic underlines how essential it is to further establish and secure global healthcare as well as to promote and extend robust research against infectious diseases.

ACKNOWLEDGMENTS

The authors are grateful to the Austrian Science Fund FWF (SFBLIPOTOX F3007&F3012, W1226, P29203, P29262, P27893) and the Austrian Federal Ministry of Education, Science and Research and the University of Graz for grants “Unkonventionelle Forschung” and “flysleep” (BMWFW-80.109/0001-WF/V/3b/2015). The authors also acknowledge the funding of DK Metabolic and Cardiovascular Disease (FWF) and the Doctoral College “Metabolic and Cardiovascular Disease” (FWFW1226) as well as support from NAWI Graz and the BioTechMed-Graz flagship

project “EPIAge”. GK is supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR) – Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; AMMICA US23/CNRS UMS3655; Association pour la recherche sur le cancer (ARC); Association “Le Cancer du Sein, Parlons-en!”; Cancéropôle Ile-de-France; Chancellerie des universités de Paris (Legs Poix), Fondation pour la Recherche Médicale (FRM); a donation by Elior; European Research Area Network on Cardiovascular Diseases (ERA-CVD, MINOTAUR); Gustave Roussy Odyssey, the European Union Horizon 2020 Project Oncobiome; Fondation Carrefour; High-end Foreign Expert Program in China (GDW20171100085), Institut National du Cancer (INCa); Inserm (HTE); Institut Universitaire de France; LeDucq Foundation; the LabEx Immunology (ANR-18-IDEX-0001); the RHU Torino Lumière; the Seerave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and the SIRIC Cancer Research and Personalized Medicine (CARPEM).

REFERENCES

- Yi Y, Lagniton PNP, Ye S, Li E, and Xu R-H (2020). COVID-19: what has been learned and to be learned about the novel coronavirus disease. *Int J Biol Sci* 16(10): 1753–1766. doi: 10.7150/ijbs.45134
- Rismanbaf A (2020). Potential Treatments for COVID-19; a Narrative Literature Review. *Arch Acad Emerg Med* 8(1): e29. PMID: 32232214
- Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, Si H-R, Zhu Y, Li B, Huang C-L, Chen H-D, Chen J, Luo Y, Guo H, Jiang R-D, Liu M-Q, Chen Y, Shen X-R, Wang X, Zheng X-S, Zhao K, Chen Q-J, Deng F, Liu L-L, Yan B, Zhan F-X, Wang Y-Y, Xiao G-F, and Shi Z-L (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579(7798): 270–273. doi: 10.1038/s41586-020-2012-7
- World Health Organization (2020). Coronavirus. Available at: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019> [Accessed: 30.04.2020]
- Guan W-J *et al.* (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. doi: 10.1056/NEJMoa2002032
- Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, Azman AS, Reich NG, and Lessler J (2020). The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Ann Intern Med* doi: 10.7326/M20-0504
- Gorbalenya AE, Baker SC, Baric RS, Groot RJ de, Drosten C, Gulyaeva AA, Haagmans BL, Lauber C, Leontovich AM, Neuman BW, Penzar D, Perlman S, Poon LLM, Samborskiy D, Sidorov IA, Sola I, and Ziebuhr J (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 5: 536–544. doi: 10.1038/s41564-020-0695-z
- Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, and Zhang L (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet Lond Engl* 395(10223): 507–513. doi: 10.1016/S0140-6736(20)30211-7
- Ren L-L *et al.* (2020). Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. *Chin Med J (Engl)*. doi: 10.1097/CM9.0000000000000722
- Velavan TP, and Meyer CG (2020). The COVID-19 epidemic. *Trop Med Int Health* 25(3): 278–280. doi: 10.1111/tmi.13383
- Schoeman D, and Fielding BC (2019). Coronavirus envelope protein: current knowledge. *Virology* 16(1): 69. doi: 10.1186/s12985-019-1182-0
- Forni D, Cagliani R, Clerici M, and Sironi M (2017). Molecular Evolution of Human Coronavirus Genomes. *Trends Microbiol* 25(1): 35–48. doi: 10.1016/j.tim.2016.09.001
- Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, Liu W, Bi Y, and Gao GF (2016). Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends Microbiol* 24(6): 490–502. doi: 10.1016/j.tim.2016.03.003
- Raoult D, Zumla A, Locatelli F, Ippolito G, and Kroemer G (2020). Coronavirus infections: Epidemiological, clinical and immunological features and hypotheses. *Cell Stress* 4(4):66-75. doi: 10.15698/cst2020.04.216
- de Wit E, van Doremalen N, Falzarano D, and Munster VJ (2016). SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 14(8): 523–534. doi: 10.1038/nrmicro.2016.81
- Ye Z-W, Yuan S, Yuen K-S, Fung S-Y, Chan C-P, and Jin D-Y (2020). Zoonotic origins of human coronaviruses. *Int J Biol Sci* 16(10): 1686–1697. doi: 10.7150/ijbs.45472
- Yin Z, Pascual C, and Klionsky DJ (2016). Autophagy: machinery and regulation. *Microb Cell* 3(12): 588–596. doi: 10.15698/mic2016.12.546
- Eisenberg T, Knauer H, Schauer A, Büttner S, Ruckstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, Fussi H, Deszcz L, Hartl R, Schraml E, Criollo A, Megalou E, Weiskopf D, Laun P, Heeren G, Breitenbach M, Grubeck-Loebenstien B, Herker E, Fahrenkrog B, Fröhlich K-U, Sinner F, Tavernarakis N, Minois N, Kroemer G, and Madeo F (2009). Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* 11(11): 1305–1314. doi: 10.1038/ncb1975
- Carmona-Gutierrez D *et al.* (2019). The flavonoid 4,4'-dimethoxychalcone promotes autophagy-dependent longevity across species. *Nat Commun* 10(1): 651. doi: 10.1038/s41467-019-08555-w

20. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, and Sinclair DA (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444(7117): 337–342. doi: 10.1038/nature05354
21. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, and Miller RA (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460(7253): 392–395. doi: 10.1038/nature08221
22. Chen G, Xie W, Nah J, Sauvat A, Liu P, Pietrocola F, Sica V, Carmona-Gutierrez D, Zimmermann A, Pendl T, Tadic J, Bergmann M, Hofer SJ, Domuz L, Lachkar S, Markaki M, Tavernarakis N, Sadoshima J, Madeo F, Kepp O, and Kroemer G (2019). 3,4-Dimethoxychalcone induces autophagy through activation of the transcription factors TFE3 and TFEB. *EMBO Mol Med* 11(11): e10469. doi: 10.15252/emmm.201910469
23. de Cabo R, Carmona-Gutierrez D, Bernier M, Hall MN, and Madeo F (2014). The search for antiaging interventions: from elixirs to fasting regimens. *Cell* 157(7): 1515–1526. doi: 10.1016/j.cell.2014.05.031
24. Levine B, and Kroemer G (2008). Autophagy in the Pathogenesis of Disease. *Cell* 132(1): 27–42. doi: 10.1016/j.cell.2007.12.018
25. Kirkin V, and Rogov VV (2019). A Diversity of Selective Autophagy Receptors Determines the Specificity of the Autophagy Pathway. *Mol Cell* 76(2): 268–285. doi: 10.1016/j.molcel.2019.09.005
26. Stolz A, Ernst A, and Dikic I (2014). Cargo recognition and trafficking in selective autophagy. *Nat Cell Biol* 16(6): 495–501. doi: 10.1038/ncb2979
27. Gatica D, Lahiri V, and Klionsky DJ (2018). Cargo recognition and degradation by selective autophagy. *Nat Cell Biol* 20(3): 233–242. doi: 10.1038/s41556-018-0037-z
28. Sharma V, Verma S, Seranova E, Sarkar S, and Kumar D (2018). Selective Autophagy and Xenophagy in Infection and Disease. *Front Cell Dev Biol* 6: 147. doi: 10.3389/fcell.2018.00147
29. Sagnier S, Daussy CF, Borel S, Robert-Hebmann V, Faure M, Blanchet FP, Beaumelle B, Biard-Piechaczyk M, and Espert L (2015). Autophagy restricts HIV-1 infection by selectively degrading Tat in CD4+ T lymphocytes. *J Virol* 89(1): 615–625. doi: 10.1128/JVI.02174-14
30. Nardacci R, Amendola A, Ciccocanti F, Corazzari M, Esposito V, Vlassi C, Taibi C, Fimia GM, Del Nonno F, Ippolito G, D’Offizi G, and Piacentini M (2014). Autophagy plays an important role in the containment of HIV-1 in nonprogressor-infected patients. *Autophagy* 10(7): 1167–1178. doi: 10.4161/auto.28678
31. Sparrner KJM, Gableske S, Zurenski MA, Parker ZM, Full F, Baumgart GJ, Kato J, Pacheco-Rodriguez G, Liang C, Pornillos O, Moss J, Vaughan M, and Gack MU (2017). TRIM23 mediates virus-induced autophagy via activation of TBK1. *Nat Microbiol* 2(11): 1543–1557. doi: 10.1038/s41564-017-0017-2
32. Yordy B, Iijima N, Huttner A, Leib D, and Iwasaki A (2012). A neuron-specific role for autophagy in antiviral defense against herpes simplex virus. *Cell Host Microbe* 12(3): 334–345. doi: 10.1016/j.chom.2012.07.013
33. Choi Y, Bowman JW, and Jung JU (2018). Autophagy during viral infection — a double-edged sword. *Nat Rev Microbiol* 16(6): 341–354. doi: 10.1038/s41579-018-0003-6
34. Cong Y, Verlhac P, and Reggiori F (2017). The Interaction between Nidovirales and Autophagy Components. *Viruses* 9(7): 182. doi: 10.3390/v9070182
35. Shi C-S, Nabar NR, Huang N-N, and Kehrl JH (2019). SARS-Coronavirus Open Reading Frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. *Cell Death Discov* 5: 101. doi: 10.1038/s41420-019-0181-7
36. Yue Y, Nabar NR, Shi C-S, Kamenyeva O, Xiao X, Hwang I-Y, Wang M, and Kehrl JH (2018). SARS-Coronavirus Open Reading Frame-3a drives multimodal necrotic cell death. *Cell Death Dis* 9(9): 904. doi: 10.1038/s41419-018-0917-y
37. Gassen NC, Niemeyer D, Muth D, Corman VM, Martinelli S, Gassen A, Hafner K, Papias J, Mösbauer K, Zellner A, Zannas AS, Herrmann A, Holsboer F, Brack-Werner R, Boshart M, Müller-Myhsok B, Drosten C, Müller MA, and Rein T (2019). SKP2 attenuates autophagy through Beclin1-ubiquitination and its inhibition reduces MERS-Coronavirus infection. *Nat Commun* 10. doi: 10.1038/s41467-019-13659-4
38. Jackson WT (2015). Viruses and the Autophagy Pathway. *Virology* 479–480: 450–456. doi: 10.1016/j.virol.2015.03.042
39. Papandreou M-E, and Tavernarakis N (2017). Autophagy and the endo/exosomal pathways in health and disease. *Biotechnol J* 12(1): 1600175. doi: 10.1002/biot.201600175
40. Gosert R, Kanjanahaluethai A, Egger D, Bienz K, and Baker SC (2002). RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. *J Virol* 76(8): 3697–3708. doi: 10.1128/jvi.76.8.3697-3708.2002
41. Lundin A, Dijkman R, Bergström T, Kann N, Adamiak B, Hannoun C, Kindler E, Jónsdóttir HR, Muth D, Kint J, Forlenza M, Müller MA, Drosten C, Thiel V, and Trybala E (2014). Targeting membrane-bound viral RNA synthesis reveals potent inhibition of diverse coronaviruses including the middle East respiratory syndrome virus. *PLoS Pathog* 10(5): e1004166. doi: 10.1371/journal.ppat.1004166
42. Knoop K, Kikkert M, Worm SHE van den, Zevenhoven-Dobbe JC, van der Meer Y, Koster AJ, Mommaas AM, and Snijder EJ (2008). SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. *PLoS Biol* 6(9): e226. doi: 10.1371/journal.pbio.0060226
43. Prentice E, Jerome WG, Yoshimori T, Mizushima N, and Denison MR (2004). Coronavirus replication complex formation utilizes components of cellular autophagy. *J Biol Chem* 279(11): 10136–10141. doi: 10.1074/jbc.M306124200
44. Prentice E, McAuliffe J, Lu X, Subbarao K, and Denison MR (2004). Identification and Characterization of Severe Acute Respiratory Syndrome Coronavirus Replicase Proteins. *J Virol* 78(18): 9977–9986. doi: 10.1128/JVI.78.18.9977-9986.2004
45. Zhu L, Mou C, Yang X, Lin J, and Yang Q (2016). Mitophagy in TGEV infection counteracts oxidative stress and apoptosis. *Oncotarget* 7(19): 27122–27141. doi: 10.18632/oncotarget.8345
46. Guo X, Zhang M, Zhang X, Tan X, Guo H, Zeng W, Yan G, Memon AM, Li Z, Zhu Y, Zhang B, Ku X, Wu M, Fan S, and He Q (2017). Porcine Epidemic Diarrhea Virus Induces Autophagy to Benefit Its Replication. *Viruses* 9(3): E53. doi: 10.3390/v9030053
47. Reggiori F, Monastyrska I, Verheije MH, Cali T, Ulasli M, Bianchi S, Bernasconi R, de Haan CAM, and Molinari M (2010). Coronaviruses Hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication. *Cell Host Microbe* 7(6): 500–508. doi: 10.1016/j.chom.2010.05.013
48. Zhao Z, Thackray LB, Miller BC, Lynn TM, Becker MM, Ward E, Mizushima NN, Denison MR, and Virgin HW (2007). Coronavirus replication does not require the autophagy gene ATG5. *Autophagy* 3(6): 581–585. doi: 10.4161/auto.4782
49. Schneider M, Ackermann K, Stuart M, Wex C, Protzer U, Schätzl HM, and Gilch S (2012). Severe acute respiratory syndrome coronavirus replication is severely impaired by MG132 due to proteasome-

- independent inhibition of M-calpain. **J Virol** 86(18): 10112–10122. doi: 10.1128/JVI.01001-12
50. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, Onderwater JJM, van der Meulen J, Koerten HK, and Mommaas AM (2006). Ultrastructure and Origin of Membrane Vesicles Associated with the Severe Acute Respiratory Syndrome Coronavirus Replication Complex. **J Virol** 80(12): 5927–5940. doi: 10.1128/JVI.02501-05
51. Guo L, Yu H, Gu W, Luo X, Li R, Zhang J, Xu Y, Yang L, Shen N, Feng L, and Wang Y (2016). Autophagy Negatively Regulates Transmissible Gastroenteritis Virus Replication. **Sci Rep** 6: 23864. doi: 10.1038/srep23864
52. Ko S, Gu MJ, Kim CG, Kye YC, Lim Y, Lee JE, Park B-C, Chu H, Han SH, and Yun C-H (2017). Rapamycin-induced autophagy restricts porcine epidemic diarrhea virus infectivity in porcine intestinal epithelial cells. **Antiviral Res** 146: 86–95. doi: 10.1016/j.antiviral.2017.08.010
53. Dong X, and Levine B (2013). Autophagy and viruses: adversaries or allies? **J Innate Immun** 5(5): 480–493. doi: 10.1159/000346388
54. Heckmann BL, and Green DR (2019). LC3-associated phagocytosis at a glance. **J Cell Sci** 132(5). doi: 10.1242/jcs.222984
55. Grose C, and Klionsky DJ (2016). Alternative autophagy, brefeldin A and viral trafficking pathways. **Autophagy** 12(9): 1429–1430. doi: 10.1080/15548627.2016.1203489
56. Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, Kanaseki T, Komatsu M, Otsu K, Tsujimoto Y, and Shimizu S (2009). Discovery of Atg5/Atg7-independent alternative macroautophagy. **Nature** 461(7264): 654–658. doi: 10.1038/nature08455
57. Niso-Santano M, Malik SA, Pietroccola F, Bravo-San Pedro JM, Mariño G, Cianfanelli V, Ben-Younès A, Troncoso R, Markaki M, Sica V, Izzo V, Chaba K, Bauvy C, Dupont N, Kepp O, Rockenfeller P, Wolinski H, Madoe F, Lavandero S, Codogno P, Harper F, Pierron G, Tavernarakis N, Ceconi F, Maiuri MC, Galluzzi L, and Kroemer G (2015). Unsaturated fatty acids induce non-canonical autophagy. **EMBO J** 34(8): 1025–1041. doi: 10.15252/embj.201489363
58. Kindrachuk J, Ork B, Hart BJ, Mazur S, Holbrook MR, Frieman MB, Traynor D, Johnson RF, Dyall J, Kuhn JH, Olinger GG, Hensley LE, and Jahrling PB (2015). Antiviral potential of ERK/MAPK and PI3K/AKT/mTOR signaling modulation for Middle East respiratory syndrome coronavirus infection as identified by temporal kinome analysis. **Antimicrob Agents Chemother** 59(2): 1088–1099. doi: 10.1128/AAC.03659-14
59. Cottam EM, Maier HJ, Manifava M, Vaux LC, Chandra-Schoenfelder P, Gerner W, Britton P, Ktistakis NT, and Wileman T (2011). Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. **Autophagy** 7(11): 1335–1347. doi: 10.4161/auto.7.11.16642
60. Cottam EM, Whelband MC, and Wileman T (2014). Coronavirus NSP6 restricts autophagosome expansion. **Autophagy** 10(8): 1426–1441. doi: 10.4161/auto.29309
61. Chan JF-W, Kok K-H, Zhu Z, Chu H, To KK-W, Yuan S, and Yuen K-Y (2020). Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. **Emerg Microbes Infect** 9(1): 221–236. doi: 10.1080/22221751.2020.1719902
62. Chen X, Wang K, Xing Y, Tu J, Yang X, Zhao Q, Li K, and Chen Z (2014). Coronavirus membrane-associated papain-like proteases induce autophagy through interacting with Beclin1 to negatively regulate antiviral innate immunity. **Protein Cell** 5(12): 912–927. doi: 10.1007/s13238-014-0104-6
63. Gusho E, Baskar D, and Banerjee S (2016). New advances in our understanding of the “unique” RNase L in host pathogen interaction and immune signaling. **Cytokine**. doi: 10.1016/j.cyto.2016.08.009
64. Yang Y, Zhang L, Geng H, Deng Y, Huang B, Guo Y, Zhao Z, and Tan W (2013). The structural and accessory proteins M, ORF 4a, ORF 4b, and ORF 5 of Middle East respiratory syndrome coronavirus (MERS-CoV) are potent interferon antagonists. **Protein Cell** 4(12): 951–961. doi: 10.1007/s13238-013-3096-8
65. Ambjørn M, Ejlerskov P, Liu Y, Lees M, Jäättelä M, and Issazadeh-Navikas S (2013). IFNβ1/interferon-β-induced autophagy in MCF-7 breast cancer cells counteracts its proapoptotic function. **Autophagy** 9(3): 287–302. doi: 10.4161/auto.22831
66. Muth D, Corman VM, Roth H, Binger T, Dijkman R, Gottula LT, Gloza-Rausch F, Balboni A, Battilani M, Rihtarič D, Toplak I, Ameneiros RS, Pfeiffer A, Thiel V, Drexler JF, Müller MA, and Drosten C (2018). Attenuation of replication by a 29 nucleotide deletion in SARS-coronavirus acquired during the early stages of human-to-human transmission. **Sci Rep** 8(1): 15177. doi: 10.1038/s41598-018-33487-8
67. Liu DX, Fung TS, Chong KK-L, Shukla A, and Hilgenfeld R (2014). Accessory proteins of SARS-CoV and other coronaviruses. **Antiviral Res** 109: 97–109. doi: 10.1016/j.antiviral.2014.06.013
68. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Seidah NG, and Nichol ST (2005). Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. **Virology** 339(2): 246–253. doi: 10.1016/j.virus.2005.07.013
69. Gautret P, Lagier J-C, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, Dupont HT, Honoré S, Colson P, Chabrière E, La Scola B, Rolain J-M, Brouqui P, and Raoult D (2020). Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. **J Antimicrob Agents** 105949. doi: 10.1016/j.ijantimicag.2020.105949
70. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W, and Xiao G (2020). Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. **Cell Res** 30(3): 269–271. doi: 10.1038/s41422-020-0282-0
71. Jaffe S (2020). Regulators split on antimalarials for COVID-19. **The Lancet** 395(10231): 1179. doi: 10.1016/S0140-6736(20)30817-5
72. Ferner RE, and Aronson JK (2020). Chloroquine and hydroxychloroquine in covid-19. **BMJ** 369: m1432. doi: 10.1136/bmj.m1432
73. Gbinigie K, and Frie K (2020). Should chloroquine and hydroxychloroquine be used to treat COVID-19? A rapid review. **BJGP Open**. doi: 10.3399/bjgpopen20X101069
74. Mauthe M, Orhon I, Rocchi C, Zhou X, Luhr M, Hijlkema K-J, Coppes RP, Engedal N, Mari M, and Reggiori F (2018). Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. **Autophagy** 14(8): 1435–1455. doi: 10.1080/15548627.2018.1474314
75. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu N-H, Nitsche A, Müller MA, Drosten C, and Pöhlmann S (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. **Cell** 181(2): 271–280.e8. doi: 10.1016/j.cell.2020.02.052
76. Al-Bari MdAA (2017). Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. **Pharmacol Res Perspect** 5(1): e00293. doi: 10.1002/prp2.293
77. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, and Veasler D (2020). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. **Cell** 181(2): 281–292.e6. doi: 10.1016/j.cell.2020.02.058
78. Zhao X, Guo F, Comunale MA, Mehta A, Sehgal M, Jain P, Cuconati A, Lin H, Block TM, Chang J, and Guo J-T (2015). Inhibition of endoplasmic reticulum-resident glucosidases impairs severe acute respiratory syndrome coronavirus and human coronavirus NL63 spike pro-

- tein-mediated entry by altering the glycan processing of angiotensin I-converting enzyme 2. **Antimicrob Agents Chemother** 59(1): 206–216. doi: 10.1128/AAC.03999-14
79. Wu K, Zhang Q, Wu X, Lu W, Tang H, Liang Z, Gu Y, Song S, Ayon RJ, Wang Z, McDermott KM, Balistreri A, Wang C, Black SM, Garcia JGN, Makino A, Yuan JX-J, and Wang J (2017). Chloroquine is a potent pulmonary vasodilator that attenuates hypoxia-induced pulmonary hypertension. **Br J Pharmacol** 174(22): 4155–4172. doi: 10.1111/bph.13990
80. Martina JA, Diab HI, Lishu L, Jeong-A L, Patange S, Raben N, and Puertollano R (2014). The Nutrient-Responsive Transcription Factor TFE3, Promotes Autophagy, Lysosomal Biogenesis, and Clearance of Cellular Debris. **Sci Signal** 7(309): ra9. doi: 10.1126/scisignal.2004754
81. Roczniak-Ferguson A, Petit CS, Froehlich F, Qian S, Ky J, Angarola B, Walther TC, and Ferguson SM (2012). The transcription factor TFE3 links mTORC1 signaling to transcriptional control of lysosome homeostasis. **Sci Signal** 5(228): ra42. doi: 10.1126/scisignal.2002790
82. Gordon DE et al. (2020). A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing. **bioRxiv [Preprint]**. Version 3, posted March 27, 2020. doi: 10.1101/2020.03.22.002386
83. Le Sage V, Cinti A, Amorim R, and Moulard AJ (2016). Adapting the Stress Response: Viral Subversion of the mTOR Signaling Pathway. **Viruses** 8(6): 152. doi: 10.3390/v8060152
84. Ranadheera C, Coombs KM, and Kobasa D (2018). Comprehending a Killer: The Akt/mTOR Signaling Pathways Are Temporally Hijacked by the Highly Pathogenic 1918 Influenza Virus. **EBioMedicine** 32: 142–163. doi: 10.1016/j.ebiom.2018.05.027
85. Gassen NC, Papies J, Bajaj T, Dethloff F, Emanuel J, Weckmann K, Heinz DE, Heinemann N, Lennarz M, Richter A, Niemeyer D, Corman VM, Giavalisco P, Drosten C, and Muller MA (2020). Analysis of SARS-CoV-2-controlled autophagy reveals spermidine, MK-2206, and niclosamide as putative antiviral therapeutics. **bioRxiv [Preprint]**. Version 1, posted April 15, 2020. doi: 10.1101/2020.04.15.997254
86. Pegg AE (2016). Functions of Polyamines in Mammals. **J Biol Chem** 291(29): 14904–14912. doi: 10.1074/jbc.R116.731661
87. Madeo F, Eisenberg T, Pietrocola F, and Kroemer G (2018). Spermidine in health and disease. **Science** 359(6374): eaan2788. doi: 10.1126/science.aan2788
88. Eisenberg T et al. (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. **Nat Med** 22(12): 1428–1438. doi: 10.1038/nm.4222
89. Morselli E, Mariño G, Bennetzen MV, Eisenberg T, Megalou E, Schroeder S, Cabrera S, Bénit P, Rustin P, Criollo A, Kepp O, Galluzzi L, Shen S, Malik SA, Maiuri MC, Horio Y, López-Otín C, Andersen JS, Tavernarakis N, Madeo F, and Kroemer G (2011). Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome. **J Cell Biol** 192(4): 615–629. doi: 10.1083/jcb.201008167
90. Gupta VK, Scheunemann L, Eisenberg T, Mertel S, Bhukel A, Koemans TS, Kramer JM, Liu KSY, Schroeder S, Stunnenberg HG, Sinner F, Magnes C, Pieber TR, Dipt S, Fiala A, Schenck A, Schwaerzel M, Madeo F, and Sigrist SJ (2013). Restoring polyamines protects from age-induced memory impairment in an autophagy-dependent manner. **Nat Neurosci** 16(10): 1453–1460. doi: 10.1038/nn.3512
91. Sonawane ND, Szoka FC, and Verkman AS (2003). Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. **J Biol Chem** 278(45): 44826–44831. doi: 10.1074/jbc.M308643200
92. Mounce BC, Olsen ME, Vignuzzi M, and Connor JH (2017). Polyamines and Their Role in Virus Infection. **Microbiol Mol Biol Rev** 81(4): e00029-17. doi: 10.1128/MMBR.00029-17
93. Mounce BC, Poirier EZ, Passoni G, Simon-Loriere E, Cesaro T, Prot M, Stapleford KA, Moratorio G, Sakuntabhai A, Levraud J-P, and Vignuzzi M (2016). Interferon-Induced Spermidine-Spermine Acetyltransferase and Polyamine Depletion Restrict Zika and Chikungunya Viruses. **Cell Host Microbe** 20(2): 167–177. doi: 10.1016/j.chom.2016.06.011
94. Zhu Y, Deng J, Nan M-L, Zhang J, Okeunle A, Li J-Y, Yu X-Q, and Wang P-H (2019). The Interplay Between Pattern Recognition Receptors and Autophagy in Inflammation. **Adv Exp Med Biol** 1209: 79–108. doi: 10.1007/978-981-15-0606-2_6
95. Akira S, Uematsu S, and Takeuchi O (2006). Pathogen recognition and innate immunity. **Cell** 124(4): 783–801. doi: 10.1016/j.cell.2006.02.015
96. Dengjel J, Schoor O, Fischer R, Reich M, Kraus M, Müller M, Kreyemborg K, Altenberend F, Brandenburg J, Kalbacher H, Brock R, Driessen C, Rammensee H-G, Stevanovic S, and Cresswell P (2005). Autophagy Promotes MHC Class II Presentation of Peptides from Intracellular Source Proteins. **Proc Natl Acad Sci U S A** 102(22): 7922–7927. doi: 10.1073/pnas.0501190102
97. Münz C (2016). Autophagy Beyond Intracellular MHC Class II Antigen Presentation. **Trends Immunol** 37(11): 755–763. doi: 10.1016/j.it.2016.08.017
98. English L, Chemali M, Duron J, Rondeau C, Laplante A, Gingras D, Alexander D, Leib D, Norbury C, Lippé R, and Desjardins M (2009). Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. **Nat Immunol** 10(5): 480–487. doi: 10.1038/ni.1720
99. Jiang G-M, Tan Y, Wang H, Peng L, Chen H-T, Meng X-J, Li L-L, Liu Y, Li W-F, and Shan H (2019). The relationship between autophagy and the immune system and its applications for tumor immunotherapy. **Mol Cancer** 18(1): 17. doi: 10.1186/s12943-019-0944-z
100. Pietrocola F et al. (2016). Caloric Restriction Mimetics Enhance Anticancer Immunosurveillance. **Cancer Cell** 30(1): 147–160. doi: 10.1016/j.ccell.2016.05.016
101. Puleston DJ, Zhang H, Powell TJ, Lipina E, Sims S, Panse I, Watson AS, Cerundolo V, Townsend AR, Klenerman P, and Simon AK (2014). Autophagy is a critical regulator of memory CD8+ T cell formation. **eLife** 3. doi: 10.7554/eLife.03706
102. Zhang H, Alsaleh G, Feltham J, Sun Y, Napolitano G, Riffelmacher T, Charles P, Frau L, Hublitz P, Yu Z, Mohammed S, Ballabio A, Balabanov S, Mellor J, and Simon AK (2019). Polyamines Control eIF5A Hypusination, TFE3 Translation, and Autophagy to Reverse B Cell Senescence. **Mol Cell** 76(1): 110-125.e9. doi: 10.1016/j.molcel.2019.08.005
103. Harris J, Lang T, Thomas JPW, Sukkar MB, Nabar NR, and Kehrl JH (2017). Autophagy and inflammasomes. **Mol Immunol** 86: 10–15. doi: 10.1016/j.molimm.2017.02.013
104. Ponpuak M, Mandell MA, Kimura T, Chauhan S, Cleary C, and Deretic V (2015). Secretory autophagy. **Curr Opin Cell Biol** 35: 106–116. doi: 10.1016/j.cceb.2015.04.016
105. Delorme-Axford E, Donker RB, Mouillet J-F, Chu T, Bayer A, Ouyang Y, Wang T, Stolz DB, Sarkar SN, Morelli AE, Sadovsky Y, and Coyne CB (2013). Human placental trophoblasts confer viral resistance to recipient cells. **Proc Natl Acad Sci U S A** 110(29): 12048–12053. doi: 10.1073/pnas.1304718110
106. Ahmad L, Mashbat B, Leung C, Brookes C, Hamad S, Krokowski S, Shenoy AR, Lorenzo L, Levin M, O'Hare P, Zhang S-Y, Casanova J-L, Mostowy S, and Sancho-Shimizu V (2019). Human TANK-binding ki-

nase 1 is required for early autophagy induction upon herpes simplex virus 1 infection. **J Allergy Clin Immunol** 143(2): 765-769.e7. doi: 10.1016/j.jaci.2018.09.013

107. Kepp O, Chen G, Carmona-Gutierrez D, Madeo F, and Kroemer G (2020). A discovery platform for the identification of caloric restriction

mimetics with broad health-improving effects. **Autophagy** 16(1): 188–189. doi: 10.1080/15548627.2019.1688984

108. Madeo F, Carmona-Gutierrez D, Hofer SJ, and Kroemer G (2019). Caloric Restriction Mimetics against Age-Associated Disease: Targets, Mechanisms, and Therapeutic Potential. **Cell Metab** 29(3): 592–610. doi: 10.1016/j.cmet.2019.01.018