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SARS-Cov-2 spike protein fragment 674–685 protects mitochondria from releasing cytochrome c in response to apoptogenic influence

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1. Introduction

Covid-19 has changed the life style of people all over the world by causing dozens of millions of illness cases and millions of deaths. During the last year multiple studies were performed to understand the peculiarities of SARS-Cov-2 infection causing the disease and to develop specific drugs and vaccines to cure it. However, many aspects of virus interaction with target cells and its consequences are still poorly understood. Covid-19 causes pathological impairment of many organs and tissues, the main clinical symptom being a severe acute respiratory syndrome (SARS). It is believed that the virus infects cells after interaction with angiotensin-converting enzyme-2 (ACE-2) receptor expressed, in particular, in respiratory epithelium [1]. Unexpectedly, smoking, which strongly affects respiratory system, appeared not to be a risk factor for severe Covid-19. Moreover, clinical observations have shown that smoking people were relatively rare among hospitalized Covid-19 patients [2]. These data attracted attention to a possible relation of SARS-Cov-2 virus to nicotinic acetylcholine receptors (nAChRs) underlying smoking addiction and nicotine effects. Structural studies have demonstrated that the fragment 674–685 of SARS-Cov-2 spike protein is homologous to the fragment 27–37 of α-cobratoxin underlying its interaction with α7 nicotinic acetylcholine receptors (nAChRs). Further in silico studies predicted a direct interaction of this SARS-Cov-2 spike protein fragment with the portion 179–190 of α7 nAChR [6].

The α7 nAChRs are involved in regulating many physiological processes including inflammation [7], memory and behavior [8], immune response [9] and blood coagulation [10]. Since all these processes seem to be impaired upon SARS-Cov-2 infection, the available data suggested an important role of SARS-Cov-2 - α7 nAChR interaction in Covid-19 pathogenesis and proposed α7-specific drugs as therapeutic tools to cure the infected patients [3,4,11]. In addition to the cell plasma membrane, the α7 nAChRs are expressed in the outer membrane of mitochondria and are involved in regulating the early events of mitochondria-driven apoptosis [12,13]. In the present paper, we put an aim to study the interaction of SARS-Cov-2 spike protein peptide 674–685 with α7 nAChR and to elucidate if such interaction influences mitochondria. The data presented indicate for the first time that SARS-Cov-2 spike protein peptide can prevent the development of
mitochondria-driven apoptosis by attenuating cytochrome c release from mitochondria.

2. Materials and methods

2.1. Cells and reagents

All reagents and antibodies against VDAC1 and α-lamin 1B were purchased from Sigma-Aldrich (Saint Louis, USA). The peptides corresponding to SARS-Cov-2 spike protein fragment 674–685, α-cobratoxin fragment 27–37 and α3 nACHr subunit fragment 11–23 were synthesized by JPT Peptide Technologies GmbH (Berlin, Germany). Antibodies against α7(1–208) [14], α7(179–190) and α4(181–192) [15] nACHr fragments and against cytochrome c [16] were obtained, validated and biotinylated previously in our lab. Mitochondria isolation kit, Bax-specific and IRE-1α-specific antibodies and Neutravidin-peroxidase conjugate were purchased from Invitrogen and were purchased from ALT Ukraine Ltd (representative of Thermo Fisher Scientific in Ukraine).

U373 cells (ATCC HTB-17) were from the stocks of Palladin Institute of Biochemistry. They were grown in 75 cm² flasks at 37 °C in RPMI1640 medium supplemented with 20 mM HEPES, 100 units/ml penicillin-streptomycin mixture and 10% FCS. Before the experiment, the cells were detached from the flask bottoms by gentle scratching in 0.2% Versene solution, washed by centrifugation, counted and resuspended in the culture medium.

2.2. Animals

We used female C57BL/6J mice, 2–5 months of age, 20–25 g of weight. Animals were kept in the animal facility of Palladin Institute of Biochemistry. They were housed in quiet, temperature-controlled rooms and were provided with water and food pellets ad libitum. Before removing the brain mice were sacrificed by cervical dislocation. All procedures conformed to the guidelines of Palladin Institute’s IACUC. Before starting the experiments, the protocols were approved by the IACUC.

2.3. Mitochondria isolation

Mitochondria were isolated from mouse brain by differential ultracentrifugation according to standard published procedures [12] and from U373 cells according to instructions of the kit manufacturer. Their purity was characterized by ELISA using the antibodies against mitochondria-specific voltage-dependent anion channel (VDAC1 [17]), nuclear-specific marker α-lamin B1 [18] and endoplasmic reticulum-specific marker IRE-1α [19] as described previously [20].

2.4. Antibody binding assay

To prepare detergent lysates, the pellets of cells or mitochondria were frozen at −20 °C, thawed and treated with lysing buffer (0.01 M Tris-HCl, pH 8.0; 0.14 mM NaCl; 0.025% NaN₃; 1% Tween-20 and protease inhibitors cocktail) for 2 h on ice upon intensive stirring. The resulting lysates were pelleted by centrifugation (20 min at 20,000 g). The protein concentration was determined with the BCA Protein Assay kit (Thermo Scientific, Rockford, USA).

The immunoplates (Nunc, Maxisorp) were coated with rabbit α7(1–208)-specific antibody (20 μg/ml), blocked with 1% BSA, and the detergent lysates of cells or mitochondria (80–100 μg/ml) were applied into the wells for 2 h at 37 °C. The plates were washed with water and were pre-incubated with SARS-Cov-2 S-protein peptide, α-cobratoxin peptide or α3 peptide for 15 min at RT. The second biotinylated α4(181–192)-specific antibody or α7(179–190)-specific antibody were applied for additional 2 h being revealed with Neutravidin-peroxidase conjugate and o-phenylenediamine-containing substrate solution. The optimal second antibody concentration (4 nM) was established in previous experiments. The peptide concentrations corresponded to the molar ratios of 1:1, 5:1 and 10:1 related to the second antibody concentration assuming the molecular weights of SARS-Cov-2 spike protein-derived peptide 674–685 being 1.478 kDa, of α-cobratoxin fragment 27–37 being 1.215 kDa and of α3 nACHr subunit fragment 11–23 being 1.430 kDa.

2.5. Cytochrome c release assay

Mitochondria isolated from mouse brain were resuspended in the incubation medium containing 10 mM HEPES, 125 mM KCl, 25 mM NaCl, 5 mM sodium succinate, and 0.1 mM Pi(K), pH 7.4 and were incubated with 0.5 mM H2O2 in the presence or absence of SARS-Cov-2 spike protein-derived peptide, α-cobratoxin peptide, α3 peptide or PNU282987 (10 nM, 50 nM and 100 nM each) at room temperature, for 5 min, and immediately pelleted by centrifugation (10 min, 7000 g at 4 °C). The mitochondria supernatants were collected and tested for the presence of cytochrome c by sandwich assay as described [16]. Briefly, the immunoplates were coated with protein A-purified rabbit cytochrome c-specific IgG (40 μg/ml) and were incubated with mitochondria supernatants (undiluted) for 2 h at room temperature. The bound cytochrome c was revealed with biotinylated cytochrome c-specific IgG (20 μg/ml, 1 h at room temperature) followed by Neutravidin-peroxidase conjugate and o-phenylenediamine-containing substrate solution.

2.6. Experiments with U373 cells

1) U373 cells were incubated with 1 mM H2O2 for 1 h, washed by centrifugation and mitochondria were isolated using mitochondria isolation kit. Mitochondria were further divided into equal portions (each one originating from 10⁷ cells) and were either treated or not with PNU282987 (30 nM), SARS spike protein-derived peptide (50 nM) or α3 peptide (100 nM) for 15 min. Then mitochondria were pelleted by centrifugation, frozen, lysed and the resultant lysates were analyzed for the levels of α7-Bax and α7-VDAC1 complexes by Sandwich ELISA. Such an approach is analogous to Western blotting: the α7-containing complexes are captured (immunoprecipitated) with α7(1–208)-specific antibody (20 μg/ml) and are revealed with Bax-(4 μg/ml) or VDAC1-specific antibody (3 μg/ml). However, in contrast to immunoblot where potential non-covalent protein complexes are destroyed in SDS-treated samples, Sandwich ELISA allows measuring such complexes and comparing their levels upon mitochondria treatments. Cytochrome c was measured as described above using mitochondria detergent lysate instead of the supernatant.

2) U373 cells were seeded into 96-well plates (4 x 10³ cells per well) and cultured with 0.1 mM H2O2 in the presence or absence of 670 nM SARS spike protein peptide, CTX peptide or α3 peptide for 24 h. The peptides were added to the cells 40 min prior to H2O2. The cells viability was assessed by MTT assay [21].

2.7. Statistical analysis

All experimental schemes were repeated at least 3 times, all giving similar results. ELISAs have been performed in triplicates, MTT assay in 5 repeats. Either raw or normalized mean values were used for statistical analysis using one-way ANOVA test and Origin 9.0 software. The data are presented as Mean ± SD. p < 0.05 was
considered a significant difference.

3. Results

To elucidate if SARS-Cov-2 peptide 674–685 (further mentioned as the SARS spike protein-derived peptide) binds to α7 nAChRs we used a competitive ELISA assay in which the peptide competed with the antibody elicited against (179–190) fragment of α7 subunit for the binding to the α7-containing cell preparations. The 27–37 fragment of α-cobratoxin (further referred as CTX peptide) was used as a positive control, while the peptide of α3 nAChR subunit possessing no structural homology with CTX-peptide or SARS spike protein-derived peptide (Fig. 1) was applied as a negative control.

As shown in Fig. 2A, both CTX peptide and the SARS spike protein-derived peptide, but not α3 peptide inhibited the α7(179–190)-specific antibody binding to U373 cell detergent lysate. In contrast, none of these peptides inhibited the binding of α4(181–192)-specific antibody, which recognizes corresponding peptide fragment of α4 nAChR subunit (Fig. 2B). This kind of experiment was repeated in several α7 nAChR-expressing human cells and cell lines (DAUDI, NHA-TS, platelets) all giving similar results (data not shown). The data obtained clearly indicated that the SARS spike protein-derived peptide competes with α7(179–190)-specific antibody for the binding to α7-containing cell preparations and, therefore, is able to interact with α7 nAChRs.

The α7 nAChRs expressed in mitochondria outer membrane regulate the opening/formation of mitochondrial channel responsible for the release of pro-apoptotic substances like cytochrome c: it was shown that α7-selective agonists or type 2 positive allosteric modulators attenuate cytochrome c release from mitochondria stimulated by Ca^{2+} or H_{2}O_{2} [22]. To find out if SARS spike protein-derived peptide affects mitochondrial α7 nAChRs we tested its binding to mouse brain mitochondria followed by the assay of cytochrome c release. As shown in Fig. 3A, both CTX peptide and SARS spike protein-derived peptide, but not α3 peptide competed with α7(179–190)-specific antibody for the binding to mouse brain mitochondria preparation. Either CTX peptide or SARS spike protein-derived peptide inhibited cyt c release from mitochondria similarly to α7-specific agonist PNU282987, while α3 peptide provided no effect (Fig. 3B). Therefore, binding of SARS spike protein-derived peptide to mitochondrial α7 nAChRs attenuated cytochrome c release stimulated by H_{2}O_{2}.

Cytochrome c is released from mitochondria through a specific channel formed in the outer membrane with the participation of voltage-dependent anion channel (VDAC1) and the pro-apoptotic Bcl-2 family protein Bax [23,24]. Recently we reported that mitochondrial α7 nAChRs play an important role in molecular interactions resulting in this channel formation. Incubation of U373 cells with H_{2}O_{2} for 1 h stimulated translocation of α7 nAChRs and Bax to mitochondria and formation of α7-Bax complexes accompanied by disruption of α7-VDAC1 complexes and cytochrome c release. Incubating isolated mitochondria of H_{2}O_{2}-treated cells with PNU282987 disrupted α7-Bax complexes, returned α7 nAChRs to complexes with VDAC1 and attenuated cytochrome c release [25]. Here we asked if SARS spike protein-derived peptide affects mitochondria of U373 cells similarly to PNU282987. As shown in Fig. 4A, either PNU282987 or SARS spike protein-derived peptide, but not α3 peptide, disrupted α7-Bax complexes formed in response to H_{2}O_{2}, restored α7-VDAC1 complexes destroyed by H_{2}O_{2} and prevented cytochrome c leakage from mitochondria of H_{2}O_{2}-treated U373 cells. However, none of the peptides prevented U373 cell death after incubation with H_{2}O_{2} for 24 h (Fig. 4B).

4. Discussion

The data presented here demonstrate that SARS spike protein-derived peptide 674–685 is able to bind α7 nAChRs found in either α7-expressing cells or mitochondria and to prevent the formation of mitochondrial channel necessary for release of pro-apoptotic signals like cytochrome c. However, when added to intact cells, the SARS spike protein-derived peptide does not protect against apoptogenic effect of H_{2}O_{2}.

![Fig. 2. The α7(179–190)-specific antibody (A) or α4(181–192)-specific antibody (B) binding to U373 cell preparation in ELISA in the presence or absence of SARS-Cov-2 spike protein-derived peptide, CTX peptide or α3 peptide. Each point corresponds to M±SD of triplicate measurements; ** - p < 0.005; *** - p < 0.0005 compared to the value obtained in the absence of competing peptide. The immunoplates were coated with rabbit α7(1–208)-specific antibody (20 μg/ml), blocked with 1% BSA, and the detergent lysates of cells (80 μg/mg) were applied into the wells for 2 h at 37 °C. The plates were washed with water and were pre-incubated with SARS-Cov-2 spike protein-derived peptide, α-cobratoxin peptide or α3 peptide for 15 min at RT. The second biotinylated α4(181–192)-specific antibody or α7(179–190)-specific antibody was applied for additional 2 h being revealed with Neutravidin-peroxidase conjugate and o-phenylenediamine-containing substrate solution. The peptide concentrations corresponded to the molar ratios of 1:1, 5:1 and 10:1 related to the second antibody concentration (4 nM) assuming the molecular weights of SARS-Cov-2 S-protein-derived peptide 674–685 being 1.478 kDa, of α-cobratoxin fragment 27–37 being 1.215 kDa and of α3 nAChR subunit fragment 11–23 being 1.430 kDa.](attachment:image-url)
The presence or absence of SARS spike protein-derived, CTX, or α3 peptides, as assayed using ELISA. The peptide concentrations corresponded to the molar ratios of 1:1, 5:1 and 10:1 related to α7(179–190)-specific antibody concentration (4 nM) assuming the molecular weights of SARS-Cov-2 S-protein-derived peptide 674–685 being 1.478 kDa, of α-cobratoxin fragment 27–37 being 1.215 kDa and of α3 nAChR subunit fragment 11–23 being 1.430 kDa. Cytochrome c (Cyto c) release from isolated mitochondria was induced by 0.5 mM H₂O₂ in the presence or absence of SARS spike protein-derived, CTX, α3 peptides or PNU282987 (10, 50 or 100 nM each) and was measured by Sandwich ELISA as described in Methods. Each data point in A and B corresponds to means ± SD of triplicate measurements; **p < 0.005; ***p < 0.0005.

The ability of SARS spike protein-derived peptide and CTX peptide to bind α7 nAChRs is probably due to the presence of positively charged cluster of amino acid residues: RGKR in CTX peptide and RRAR in SARS spike protein-derived peptide, which mimic the quaternary nitrogen of choline (but not acetylcholine, because the peptides did not bind to α4-containing nAChRs, which require acetylcholine for activation). Then, the SARS-Cov-2 spike protein containing this motif can influence the function of α7 nAChRs upon infection. A fragment containing CTX-like motif (RGKR) is also present in the glycoprotein of rabies virus and was suggested to be the reason for behavioral alterations caused by rabies infection [26]. The α7 nAChRs are expressed in many organs and tissues including central nervous system, respiratory epithelium, vascular endothelium, skin and blood cells. Interaction of SARS-Cov-2 with α7 nAChRs could facilitate virus intervention and explain multiple pathological effects observed upon Covid-19. Here we show for the first time that SARS spike protein-derived peptide can influence α7 nAChRs expressed in mitochondria to inhibit apoptosis-inducing events.

It is known that coronaviruses, including SARS-Cov, encode both pro-apoptotic and anti-apoptotic proteins and can either initiate or delay the progression of apoptosis [27,28]. In parallel, viruses affect intracellular mechanisms to prevent the infected cell death before their replication cycle is complete [29,30]. According to established model, after penetrating into the cell cytoplasm, the virus particle is uncoated to enable its RNA translation by the host cell's ribosomes [31]. Correspondingly, the coating proteins are degraded inside the cell and their fragments can influence intracellular structures including mitochondria. Our data suggest that the α7 nAChR-binding portion in SARS-Cov-2 spike protein is one of molecular tools to attenuate apoptosis and support the infected cell viability in the course of SARS-Cov-2 replication. This mechanism is efficient.
only after the virus is inside the cell and does not work if the peptide affects cell surface nACHRs.

5. Conclusion

The data presented provide the first experimental evidence for SARS-Cov-2 spike protein-derived peptide 674–685 interaction with α7 nACHRs in cells and mitochondria and demonstrate that such interaction attenuates cytochrome c release from isolated mitochondria of the brain cells. These data suggest a molecular mechanism by which SARS-Cov-2 virus supports the infected cell viability until its replication cycle is complete.

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Declaration of competing interest

The authors of the manuscript “SARS-Cov-2 Spike Protein Fragment 674–685 Protects Mitochondria from Releasing Cytochrome c in Response to Apoptogenic Influence” declare that there is no conflict of interest.

References

[1] Q. Wang, Y. Zhang, L. Wu, S. Niu, C. Song, Z. Zhang, et al., Structural and functional basis of SARS-CoV-2 entry by using human ACE2, Cell 181 (4) (2020) 894–904, e9.5.
[2] K. Farsalinos, A. Barbouni, K. Poula, R. Polosa, P. Casper, R. Naurou, Current smoking, former smoking, and adverse outcome among hospitalized COVID-19 patients: a systematic review and meta-analysis, Ther. Adv. Chronic Dis. 11 (2020). 204022320015765.
[3] K. Farsalinos, R. Naurou, J. Le Houezec, A. Barbouni, A. Tsatsakis, A. Kouras, et al., Editorial: nicotine and SARS-CoV-2: COVID-19 may be a disease of the nicotinic cholinergic system, Toxicol. Rep. 7 (2020) 658–663.
[4] J.-P. Changeux, Z. Amoura, F. Rey, M. Miyara, A nicotinic hypothesis for Covid-19 with preventive and therapeutic implications, Comptes Rendus Biol. 343 (1) (2020) 33–39.
[5] Y. Bourne, T.T. Talley, S.B. Hansen, P. Taylor, P. Marchot, Crystal structure of a Cbtx-AChBP complex reveals essential interactions between snake alpha-neurotoxins and nicotinic receptors, EMBO J. 24 (8) (2005) 1512–1522.
[6] K. Farsalinos, E. Elipoulos, D.D. Leonidas, G.E. Papadopoulos, S. Tzartos, K. Poula, Nicotinic cholinergic system and COVID-19: in silico identification of an interaction between SARS-CoV-2 and nicotinic receptors with potential therapeutic targeting implications, Int. J. Mol. Sci. 21 (16) (2020) 5807.
[7] W.J. De Jonge, L. Ullio, The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation, Br. J. Pharmacol. 151 (2007) 915–929.
[8] O.Y. Lykhmus, O.M. Kalashnyk, K.R. Uspenska, M.V. Skok, Positive allosteric modulation of alpha7 nicotinic acetylcholine receptors transiently improves memory but aggravates inflammation in LPS-treated mice, Front. Aging Neurosci. 11 (2020) 359.
[9] L. Koval, O. Lykhmus, M. Zhmakh, A. Khurschev, V. Tsetlin, E. Magrini, et al., Differential involvement of α4δ2 and α7 and α910 nicotinic acetylcholine receptors in B lymphocyte activation in vitro, Int. J. Biochem. Cell Biol. 43 (2011) 1516–524.
[10] A. Schelde, S. Thornton, P. Schloss, H. Küster, P. Bugert, Human platelets express functional α7α7-nicotinic acetylcholine receptors, Arterioscler. Thromb. Vasc. Biol. 31 (4) (2011) 928–934.
[11] Y. Tizabi, B. Getachew, R.L. Copeland, M. Asher, Nicotine and the nicotinic cholinergic system in COVID-19, FEBS J. 287 (2020) 3656–3663.
[12] G.L. Gergalova, O.Y. Lykhmus, O.M. Kalashnyk, L.M. Koval, V.O. Chernyshov, E.A. Kryukova, et al., Mitochondria express α7 nicotinic acetylcholine receptors to regulate Ca2⁺ accumulation and cytochrome c release: study on isolated mitochondria, PLoS One 7 (2) (2012), e31361.
[13] M. Skok, G. Gergalova, O. Lykhmus, O. Kalashnyk, L. Koval, S. Uspenska, Nicotinic acetylcholine receptors in mitochondria: subunit composition, function and signalling, NeuroTransmitter 3 (2016), e12590.
[14] O. Lykhmus, L. Koval, S. Pavlovych, M. Zouridakis, P. Zismanopoulos, S. Tzartos, et al., Functional effects of antibodies against non-neuronal nicotinic acetylcholine receptors, Immunol. Lett. 128 (2010) 68–73.
[15] M.V. Skok, L.P. Voitenko, S.V. Voitenko, E.Y. Lykhmus, E.N. Kalashnik, T.I. Litvin, et al., Alpha subunit composition of nicotinic acetylcholine receptors in the rat autonomic ganglia neurons as determined with subunit-specific anti-alpha (181–192) peptide antibodies, Neuroscience 93 (4) (1999) 1427–1436.
[16] G. Gergalova, O. Lykhmus, S. Komisarenko, M. Skok, α7 Nicotinic acetylcholine receptors control cytochrome c release from isolated mitochondria through kinase-mediated pathways, Int. J. Biochem. Cell Biol. 49 (2014) 26–31.
[17] M. Colombini, VDAC: the channel at the interface between mitochondria and the cytosol, Mol. Cell. Biochem. 256/257 (1–2) (2004) 107–115.
[18] Y. Gruenbaum, K.L. Wilson, A. Harel, M. Goldberg, M. Cohen, Review: nuclear lamins - structural proteins with fundamental functions, J. Struct. Biol. 129 (2–3) (2000) 313–323.
[19] Y. Chen, F. Brandizzi, IRE1: ER stress sensor and cell fate executor, Trends Cell Biol. 23 (11) (2013) 547–555.
[20] K. Uspenska, O. Lykhmus, G. Gergalova, V. Chernyshov, H.R. Arias, S. Komisarenko, et al., Nicotine facilitates nicotinic acetylcholine receptor targeting to mitochondria but makes them less susceptible to selective ligands, Neurosci. Lett. 565 (2017) 43–50.
[21] J. Carmichael, W.G. DeGriff, A.F. Gazdar, J.D. Minna, J.B. Mitchell, Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of radiosensitivity, Canc. Res. 47 (1987) 943–946.
[22] K. Uspenska, O. Lykhmus, H.R. Arias, S. Pons, U. Maskos, S. Komisarenko, M. Skok, Positive allosteric modulators of α7 or α52 nicotinic acetylcholine receptors trigger different kinase pathways in mitochondria, Int. J. Biochem. Cell Biol. 99 (2018) 226–235.
[23] L.M. Dejean, S. Martinez-Caballero, L. Guo, C. Hughes, O. Teijido, T. Ducret, et al., Oligomeric Bax is a component of the putative cytochrome c release channel MAC, mitochondrial apoptosis-induced channel, Mol. Biol. Cell 16 (5) (2005) 2442–2432.
[24] V. Shoshan-Barmatz, V. De Pinto, M. Zweckstetter, Z. Raviv, N. Keinan, N. Arbel, VDAC, a multi-functional mitochondrial protein regulating cell life and death, Mol. Asp. Med. 31 (3) (2010) 227–285.
[25] O. Kalashnyk, O. Lykhmus, K. Uspenska, M. Izmailov, S. Komisarenko, M. Skok, Mitochondrial α7 nicotinic acetylcholine receptors are displaced from complexes with VDAC1 to form complexes with Bax upon apoptosis induction, Int. J. Biochem. Cell Biol. 129 (2020), 105879.
[26] K. Hueffer, S. Khatri, S. Rideout, M.B. Harris, R.L. Papke, C. Stokes, et al., Rabies virus modiﬁes host behaviour through a snake-toxin like region of its glycoprotein that inhibits neurotransmitter receptors in the CNS, Sci. Rep. 7 (2017) 12818.
[27] Z. Ye, C.K. Wong, P. Li, Y. Xie, A SARS-CoV protein, ORF-6, induces caspase-3 mediated, ER stress and JNK-dependent apoptosis, Biochim. Biophys. Acta 1780 (2008) 1383–1387.
[28] S. Li, Y. Zhang, Z. Guan, H. Li, M. Ye, X. Chen, et al., SARS-CoV-2 triggers inﬂammatory responses and cell death through caspase-8 activation, Sig. Transduct. Target. Ther. 5 (2020) 235.
[29] J.A. Blaho, Virus infection and apoptosis (issue II) an introduction: cheating genetics host behaviour through a snake-toxin like region of its glycoprotein that inhibits neurotransmitter receptors in the CNS, Sci. Rep. 7 (2017) 12818.
[30] P. Clarke, K.L. Tyler, Apoptosis in animal models of virus-induced disease, Nat. Rev. Microbiol. 7 (2) (2009) 144–155.