MAPK Activation, P53 and Autophagy Inhibition Characterize the SARS-CoV-2 Spike Protein Induced Neurotoxicity

Antonis Kyriakopoulos[1], Greg Nigh[2], Peter A McCullough[3], and Stephanie Seneff[4]

[1]1. Director and Head of Research and Development, Nasco AD Biotechnology Laboratory, Department of Research and Development, Sachtouri 11, 18536, Piraeus, Greece.; antkyriak@gmail.com
[2]2. Naturopathic Oncologist, Immersion Health, Portland, OR 97214, USA; drnigh@immersionhealthpdx.com
[3]3. Chief Medical Advisor, Truth for Health Foundation, Tucson, AZ USA; peteramccullough@gmail.com
[4]4. Senior Research Scientist, Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA USA; seneff@csail.mit.edu

November 17, 2022

Abstract

The SARS-CoV-2 spike protein and prions use common pathogenic pathways to induce toxicity in neurons. Infectious prions activate the p38 mitogen activated protein kinase (MAPK) pathway, and SARS-CoV-2 spike proteins induce the p38 MAPK and c-Jun NH2-terminal kinase (JNK) pathways through toll-like receptor signaling, indicating the potential for similar neurotoxicity, causing prion and prion-like disease. In this review we analyze the roles of autophagy inhibition, elevated intracellular p53 levels and reduced Wild-type p53-induced phosphatase 1 (Wip1) and dual-specificity phosphatase (DUSP) expression in neurons. The pathways induced by the spike protein via toll like receptor activation induce both PrP^C upregulation and β amyloid expression. Through the spike-protein-dependent elevation of p53 levels via β amyloid metabolism, increased PrP^C expression can lead to PrP misfolding and impaired autophagy, generating prion disease. We conclude that, according to the age of the spike protein-exposed patient and the state of their cellular autophagy activity, excess sustained activity of p53 in neurons may be a catalytic factor in neurodegeneration. We conclude that neurodegeneration is in part due to intensity and duration of spike protein exposure, patient age, cellular autophagy activity, and activation, function and regulation of p53. Finally, the neurologically damaging effects can be cumulatively spike-protein dependent, whether exposure is by natural infection or, more substantially, by repeated mRNA vaccination.

Keywords: SARS-Cov-2 spike protein; mRNA vaccines; prion and prion-like diseases; p53; Wip1; autophagy; aging; senescence; COVID-19.

1. Introduction and Background

A significant percentage of patients suffering from SARS-CoV-2 develop neurological and cognitive impairments, sometimes lasting long after the infection has cleared. This condition has been named "long haul COVID disease," or simply "long COVID," also known as "PASC" (Post-Acute Sequelae of SARS CoV-2 infection). An international study quantified persistent long-COVID symptoms among 3,762 individuals following a SARS-CoV-2 infection. These authors wrote: "Memory and cognitive dysfunction, experienced by over 88% of respondents, were the most pervasive and persisting neurologic symptoms in this cohort, equally common across all ages, and with substantial impact on work and daily life. Memory and cognitive dysfunction, together with other commonly reported neuropsychiatric symptoms, may point to larger

neurological issues involving both the central and peripheral nervous system" [1]. A post-mortem study of the brains of three patients who died from severe COVID-19 showed a large number of activated microglia that were associated with overexpression of inflammatory markers, including Interleukin-1β (IL-1β) and IL-6. The authors suggested that oxidative stress induced a glial-mediated neuroinflammatory response leading to neuronal injury [2].

A growing consensus attributes these symptoms to neurotoxic effects of the spike glycoprotein, particularly the S1 subunit [3]. The receptor-binding domain of SARS-CoV-2 spike S1 protein binds to heparin and to heparin-binding proteins [4]. Idrees and Kumar wrote in their conclusion: "Our results indicate stable binding of the S1 protein to these aggregation-prone proteins which might initiate aggregation of brain protein and accelerate neurodegeneration" [4]. A study evaluating the amyloidogenic potential of the spike protein verified that the spike protein can cause amyloid-like fibrils to appear after the protein has been subjected to proteolysis. A specific segment that appeared following proteolysis, spike 194-213 (FKNIDGYFKI), was demonstrated both theoretically and experimentally to be amyloidogenic [5]. A study by Kruger *et al.* found that proteolysis resistant fibrin amyloid microclots accumulate in the blood in association with PASC, and this also suggests that the spike protein has amyloidogenic properties [6].

Direct experimental evidence of S1's toxic effects in the brain comes from studies conducted by a team of Korean researchers, published in 2022 [7]. In the experiment, S1 subunits were introduced directly into the dorsal hippocampus of mice, and it was shown that the mice subsequently suffered from anxiety-like behavior and cognitive deficits. Further experiments both *in vivo* and *in vitro* found that the effects were mediated by microglia, which became activated following exposure. The microglia released excitatory cytokines, in particular IL-1β. IL-1β expression was upregulated more than seven-fold in the hippocampi of the exposed mice. Morphologically, the microglia of the exposed mice acquired the features of reactive microglia.

In this paper, we attempt to trace the likely biological pathways by which neuronal damage occurs in response to the spike protein, particularly S1. We will argue based on the emerging literature that toll-like receptor 4 signaling is central to the destructive reaction process. An important intermediary is the MAPK cascade. MAPK comprises four distinct pathways, a) the extracellular signal regulated kinase 1 and 2 (ERK1/2), b) the ERK-big MAP kinase 1(BMK1), c) the c-Jun NH2-terminal kinases (JNK) or stress activated protein kinases (SAPKs), and d) the p38 MAPKs. The ERK pathways are stimulated by growth factors, hormones and pro-inflammatory stimuli whereas the JNK and p38 MAPK are activated by cellular and environmental stress signals in addition to pro-inflammatory stimuli [8,9]. It is these latter two pathways that we will argue play a primary role in spike protein neurotoxicity.

Recent neurotoxicity studies indicate that the SARS-CoV-2 S1 subunit induces neuro-inflammation in microglial cells, a special type of macrophage in the central nervous system (CNS) [10,11]. The neuroinflammatory response is mediated by p38 MAPK and nuclear factor ϰ-light chain enhancer of activated B cells (NF-ϰB) activation, mainly through the pattern recognition receptor TLR4. In addition, the SARS CoV-2 S1 subunit elicits a pro-inflammatory response in murine and human macrophages by activating TLR4 receptor signaling. In this signaling process, both JNK and p38 are activated by phosphorylation [12]. It is important to note that infectious prions also activate the p38 MAPK pathway to induce their neurotoxicity effects [13]. The spike protein has prion-like characteristics that may contribute to its neurotoxicity. We will return to this topic in great detail later.

2. Modifications in the Vaccine mRNA Sequence

The technology behind the mRNA vaccines is complex and sophisticated, and much about it is new and poorly evaluated for safety [14]. The mRNA in the vaccines is very different from the mRNA sequence that the virus uses to encode the spike protein. A significant modification was to replace all of the uridines in the sequence with methylpseudouridines. This allows the mRNA to resist enzymatic breakdown. It has been shown that methylpseudouridine modifications support more than ten times as much protein as unmodified mRNAs, in part by preventing repression of translation initiation [15,16].

Other ingredients include polyethylene glycol and a synthetic cationic lipid, which facilitates escape from

the lysosome into the cytoplasm and initiation of protein synthesis. The actual sequence itself is also modified, through a process called "codon optimization," which involves substituting redundant codons that translate more efficiently than the codons the virus used for each amino acid. A codon replacement that actually changes the peptide sequence is also introduced, replacing two adjacent amino acids with a double proline sequence that disrupts the refolding step to facilitate membrane entry following binding to the ACE2 receptor. Finally, the mRNA molecule is "humanized" by inserting 5' and 3' untranslated regions (UTRs)) on its two ends, sequences borrowed from long-lasting human mRNAs, and adding a long poly-A tail to further promote resistance to breakdown [14]. The spike protein shares regions of high molecular similarity with many important human proteins, and molecular mimicry may lead to autoimmune disease, especially because the vaccine induces a very strong antibody response [17].

It appears that the developers were very successful in assuring rapid synthesis of the spike protein sustained over a long period of time. Most mRNA molecules are eliminated within a few hours of their synthesis, whereas spike protein mRNA has been found in the draining lymph nodes of the arm muscle two months after vaccination, and this durability was associated with post-vaccine symptoms similar to the symptomatic profile of long COVID [18]. Fertig *et al.* have found mRNA circulating for at least two weeks after vaccination [19].

3. CD16+ Monocytes and Spike Protein Persistence

Remarkably, the spike protein has been found to persist in human CD16+ monocytes circulating in the blood as much as 15 months after infection with SARS-CoV-2 [20]. Spike persistence was associated with long COVID symptoms, and it was suggested that persistent spike presence could explain lingering symptoms. This was not reflecting an active infection, as only fragmented SARS-CoV-2 RNA was found in these PASC patients. This finding is mysterious, as 15 months seems too long for either a protein or a messenger RNA molecule to survive.

It is possible that this feat is achieved through a process that includes reverse transcription of the mRNA into DNA [21]. A recent*in vitro* study demonstrated that human liver cancer cells are able to convert the mRNA from the COVID vaccines into DNA within six hours of exposure [22]. Cancer cells are known to often express high levels of long interspersed nuclear element-1 (LINE-1), a retrotransposon that is capable of reverse transcribing mRNA into DNA. Expression of LINE-1 is higher in tumors with p53 mutations [23].

Furthermore, and remarkably, tumors release extracellular vesicles containing retroelements that can be taken up by circulating monocytes, especially under inflammatory conditions [24]. This suggests a mechanism by which the CD16+ monocytes could acquire the capability to reverse transcribe mRNA. Alternatively, tumor cells could be releasing exosomes containing mRNA coding for the spike protein, which is then taken up directly by the circulating monocytes and translated into protein [25].

Furthermore, the CD16+ monocytes themselves are likely to be long-lived. The CD16+ subset of circulating monocytes is known as the "inflammatory" subset, because they typically release higher amounts of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), and low levels of the anti-inflammatory cytokine IL-10, in response to toll-like receptor (TLR) stimulation, compared to "classical" CD16-monocytes. Normally, they make up 10-20% of the circulating monocyte pool, but their numbers are expanded in association with inflammatory conditions.

MicroRNAs are short single-stranded non-coding RNA molecules that function in post-transcriptional regulation of gene expression. MiR-146a in particular is a well-known marker for a "senescent" phenotype. The basal level of this miRNA is significantly higher in CD16+ monocytes than in the classical monocytes. Senescence is a long-lived state of irreversible proliferative arrest. The senescent monocyte remains alive for an extended period, continually releasing inflammatory cytokines [26].

Another mechanism by which the spike protein could persist long-term would be through its misfolding into a protease-resistant form. The spike protein is a glycoprotein, and glycoproteins from viruses have been shown to facilitate the spreading of proteopathic seeds. In a seminal experiment, cells propagating tau aggregates

were transfected with a vector coding for the spike protein. The S1 segment was identified in lysates of transfected cells, and also showed up in extracellular vesicles secreted by these cells. ACE2-equipped HEK cells served as recipients, and it was demonstrated that the presence of spike protein expression in the source cells significantly increased the number of recipient cells with induced aggregates [27].

In a study investigating the durability of spike protein production following vaccination, abundant spike protein was still present in germinal centers in draining lymph nodes 16 days after the second vaccine, and spike antigen was still present as late as 60 days after the second vaccine [28]. A 2022 study by Bansal et al. showed that the spike protein appeared in circulating exosomes 14 days after the first mRNA vaccine dose, and that spike-containing exosomes were still detectable four months later. They argued that these exosomes played an essential role in the induction of antibodies [29].

4. Activation of TLR4

While it is well established that the SARS-CoV-2 virus gains entry into human cells via the ACE2 receptor, there is another activation pathway that may be responsible for the cytokine storm associated with severe disease. A gene expression assay study involving peripheral blood mononuclear cells drawn from 48 subjects, including 28 COVID-19 patients (8 severe vs. 20 mild) revealed that severe cases were associated with activation of TLR4 signaling and a response that bore a strong resemblance to bacterial sepsis [30]. Furthermore, *in vitro*studies on both human and mouse macrophages demonstrated that the S1 subunit of the spike protein alone activates TLR4 receptors and induces a strong inflammatory response via the NF-ϰB and JNK pathways [12]. The spike protein has also been shown to activate TLR2 [31]. This receptor is specifically associated with induction of IL-6 [32]. A case study involved four individuals who died of an "unknown cause" following a second dose of an mRNA vaccine. RNA sequencing revealed that genes involved in neutrophil degranulation and a cytokine storm were sharply upregulated in the cases compared to controls, suggesting that the vaccines induced an excessive inflammatory response [33]. Another experiment showed that the S1 subunit of the SARS-CoV-2 spike protein interacts specifically with the extracellular leucine rich repeat domain of TLR4 to activate NF-ϰB [34].

TLR4 is a receptor that often responds to bacterial infections. The best-known stimulator of the TLR4 response is bacterial lipopolysaccharide (LPS). There is an acidic four-amino-acid sequence (PRRA) in the S1 segment of the spike protein, just above the furin cleavage site, unique among coronaviruses, that is also found in*Staph aureus* enterotoxin B (SEB), an extremely toxic enterotoxin. SEB is a potent inducer of TNF-α, and it induces an expansion of the pool of CD16+ monocytes. SARS-CoV-2 entry into cells can be inhibited by a monoclonal antibody against SEB [35,36]. It is possible that toll like receptor activation by spike depends in part on this unique sequence.

CD16+ cells are known for their more mature stage compared to other circulating monocytes. They are the primary cell type that infiltrates inflammatory tissues and launches the TLR4 signaling cascade [37].

When inflammation is occurring outside the CNS, there is a systemic response that takes place in the brain, whereby microglia become activated and upregulate TNF-α signaling. Subsequently, circulating monocytes are recruited into the brain through enhanced expression of cerebral monocyte chemoattractant protein (MCP)-1 [38]. Through such a mechanism, it is possible that CD16+ monocytes deliver spike protein to the brain, causing neuronal injury and explaining cognitive issues linked to long COVID.

A case study involved a 76-year-old man with Parkinson's disease who died three weeks after his third immunization against COVID-19 (the BNT162b2 mRNA vaccine) [39]. Histopathological analyses of the brain revealed acute lymphocytic vasculitis and multifocal necrotizing encephalitis. Immunohistochemistry analysis identified the spike protein but not the nucleocapsid protein in the foci of inflammation in both the brain and the heart. The patient had not been previously diagnosed with COVID-19, so there is strong evidence that the vaccine caused this condition.

5. The Aggregation-prone Prion Protein: Normal Function and Expression

Central to prion disease pathology are the conformational changes of the normal prion protein (PrP) isoform,

PrP^{C}, which is located primarily on the surface of nerve cells. Conformational changes on the tertiary structure of PrP^{C} result in the infectious form of the protein, also referred to as the misfolded isoform PrP^{SC} (SC stands for "scrapie," the prion disease that occurs in sheep). These misfolded proteins aggregate into long fibrils and deregulate normal functioning of the brain, leading to prion-related disease such as scrapie, Alzheimer's disease (AD), and several others [40]. The non-infectious form PrP^{C}, under non-pathogenic conditions, plays many beneficial cellular roles. It participates in lymphocyte activation, cellular differentiation, neurite outgrowth, synaptogenesis, cellular signaling and viability, cellular adhesion processes and many other important functions for cellular homeostasis (for review see Castle AR and Gill AC 2017 [41]).

Overall, PrP^{C} is a stress-induced protein offering cellular protection under stress conditions, and its normal level is increased under conditions of hypoglycemia, ischemia, and in the presence of insulin. Some of the many beneficial roles of PrP and also of β-amyloid precursor protein (APP), which is linked to Alzheimer's disease, are presented in Table 1. The expression of PrP^{C} is subject to a plethora of transcription factors that are elevated by stress-inducing cellular conditions.

Endoplasmic reticulum stress also induces PrP^{C}expression, as shown in Table 1 [41]. The Prion Protein gene (*PRNP*), although it may be regarded as a housekeeping gene, has multiple binding sites for transcription factors in its promoter region, including the selective promoter factors Sp1 and Sp2, normally known for their tumorigenicity potential [42].

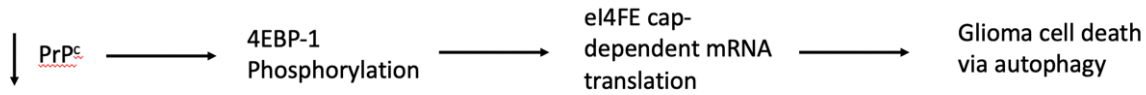
Table 1. Some of the normal prion protein and β-amyloid precursor protein physiological functions.

Prion protein (PrP^{C})	
Function	**Effects and properties**
Stress and neuroprotection	Anti-oxidative stress response [43,44] Protection from ER-stress induced apoptosis [45]
Regulation of autophagy	Supports autophagy by facilitating autophagosome-lysosomal fusion [46]
Regulation in cancer progression	Induction of cell survival in tumor cells [47]
B-amyloid precursor protein (APP)	
Stimulation of cellular growth	Proper neurite outgrowth [48]
Neural stem cells viability	Increases and sustains the proliferation of neural progenitor cells [49,50]
Regulation of synaptic plasticity, learning and memory	Supports dendritic spine formation during development [51] Enhances N-methyl-D-aspartate receptor (NMDAR) function [52]
Regulation of blood coagulation and wound repair	Accumulation in platelet α granules and release during wound healing [53] Anti-coagulant properties to regulate thrombosis after cerebral vascular injury [54]

Activating protein-1 (AP-1) and AP-2, along with a variety of dimers of the Jun and Fos family, are among several transcription factors with a high affinity for the GC-rich putative binding and promoter regions within PRNP . Activation by these transcription factors plays a regulatory function in the brain [55]. These

transcription factors are operational as a consequence of JNK activation and c-Jun phosphorylation, as well as the E4 promoter binding protein. Expression of this binding protein depends on the intracellular levels of calcium (Ca2+), together with many additional transcription factors involved in phosphatase pathway regulation [56,57].

Interestingly, in experiments which induce the antisense silencing of PrP^C expression, the result is a phosphorylation of 4E binding protein-1 (4EBP-1), a molecular event that causes the release of eukaryotic translation initiation factor 4E (eIF4E) to proceed to cap-dependent mRNA translation. This in turn causes an autophagy-dependent cell death in glioma cells [58,59]. I.e.,



Notably and relevantly, it has been shown that inducing cells to favor cap-dependent translation via the high affinity between caps of synthetic mRNAs and eIF4E drives the recipient cells toward an increased tendency for proliferation and towards initiation of the cellular events that favor oncogenesis, immune dysregulation, and aging defects. The synthetic mRNA cap currently resident on the SARS-CoV-2 mRNA used for genetic vaccination is precisely the cap composition that favors cap-dependent translation of the mRNA. Furthermore, there are at least two additional cellular factors also driving cap-dependent translation in cells stressed by the presence of the synthetic mRNA and its spike protein product. These include a) the p38 MAPK pathway and, b) the imbalance of p53 inhibitory activity toward the mechanistic target of rapamycin (mToR) axis. [60].

In summary, the mRNA vaccines currently in use brings about a constellation of circumstances that drive cells toward cap-dependent translation of that mRNA – a process with a number of expected but not well characterized detrimental effects on cellular homeostasis.

6. The Prion Protein and Autophagy

An impairment or failure of macro-autophagy is being increasingly recognized as a primary contributor to prion disease [61,62]. In a paper published in 2020 by a team of researchers in Spain, the authors wrote in the abstract: "Autophagy is now emerging as a host defense response in controlling prion infection that plays a protective role by facilitating the clearance of aggregation-prone proteins accumulated within neurons." [63]. Macro-autophagy is an important pathway by which misfolded prion protein itself is degraded, and drugs that induce autophagy have been shown to have anti-prion effects [64]. Autophagic vacuoles normally form and then fuse with endolysosomes for eventual clearance [65]. With increased autophagy activity, the neuron is less likely to release prion proteins within exosomes to induce spread of infectivity to other neurons [64]. Interestingly, the prion protein is upregulated under multiple stressed conditions, and it has been proposed that an important role it plays is to facilitate the fusion of the autophagosomes with lysosomes to promote clearance of cellular debris – including misfolded proteins and damaged mitochondria.

There exist strains of mice used in research laboratories that have a genetic mutation in the prion protein gene which disables its expression. These mice provide important knowledge about the functions of the prion protein by virtue of its absence. A key feature of these mice is the appearance very early in life of autophagic vacuoles in the cytoplasm. Vacuoles appeared as early as 3 months of age in cortical neurons, and by 6 months they had also appeared in hippocampal neurons. The number of vacuoles increased in the hippocampus at an accelerated rate with aging compared to control mice. These defective mice were more sensitive to oxidative stress, and they had an increased risk to seizures, motor and cognitive abnormalities, and impaired long-term potentiation in the hippocampus [66]. These mice provide strong support for the view that the prion protein supports autophagic clearance of cellular debris.

Curiously, autophagic vacuoles are also a common feature of neurodegenerative disease, including Creutzfeldt Jakob Disease (CJD) [62]. The facts that both too little and too much prion protein lead to similar disease states can be explained if we assume that prion disease is mainly a loss-of-function pathology. When the neuron is exposed to stressors that increase the burden of misfolded proteins, it upregulates PrP to assist in the removal of this debris via the lysosomal system. But once there are seed misfolded PrP^{SC} proteins, or externally supplied misfolded prion-like proteins such as the spike protein, along with the high concentration of PrP induced by the stressors, there is the potential for the seed to recruit most of the PrP present in the cytoplasm, converting it first to soluble oligomers and finally to precipitated fibrils. While the amount of PrP in the cell is high, most of it is tied up in the oligomers and fibrils, so it is no longer able to clear the debris, resulting in the accumulation of vacuoles.

7. The Relations of PrP^{C} and APP to Phosphorylation Pathways and Beyond

Although PrP^{C} participates in vitally important cellular functions (Table 1), the conformational conversion of PrP^{C} to PrP^{SC} is the hallmark to prion disease progression, and the prerequisite for this conversion is the expression and presence of PrP^{C}. In the absence of endogenous PrP^{C} there is an overwhelming resistance to the development of prion disease [67].

On the one hand, the role of normal isoform PrP^{C}presence seems to be protective for cells, as for example is the case where the suppression of PrP mRNA expression leads to onset of premature aging processes [66]. On the other hand, the tissues that do not express PrP^{C} are resistant to PrP^{SC}toxicity. It is the infectious PrP^{SC} which aggregates to form fibrils, and the oligomers of these fibrils that are highly infectious and neurotoxic, and it is their relations to phosphorylation pathways that constitute the pathogenesis mechanisms of prion and prion-like diseases [13,68,69]. Additionally, the intramolecular regions (tandem repeats) of tau protein strongly interact with the octapeptide repeats of wild type PrP, and more strongly with the mutant types of PrP^{SC}, to form strongly bound complexes [70]. This highlights the potential mutual involvement of both PrP^{C} and tau proteins in the context of common pathogenic mechanisms causing prion disease, as well as tau-related neurodegeneration. The prion-like propagation that ensues also involves β-amyloid protein aggregation, which induces tauopathy as it is encountered in Alzheimer's Disease (AD) [68,69,71].

Importantly, the acceleration of PrP^{SC} formation through the cellular pathways just described drives forward, in a positive-feedback manner, the initiation and progression of tau-related pathology, including the production and aggregation of tau proteins. It is within this context that the events of inter-related neurodegenerative pathogenesis mechanisms transpire. Moreover, the advance and proliferation of misfolded PrP to an at-risk human organism's neuronal tissues precedes the onset of neuro-pathogenesis disease mechanisms, suggesting that PrP^{C}over-expression is a major contributor to the onset of prion and prion-like diseases [68].

Protein aggregation is common in some neurodegenerative diseases, such as AD, Parkinson's Disease (PD) and Huntington's Disease (HD). However, another common characteristic of prion and prion-like diseases is the improper conformation alignment of their disease-related proteins, i.e., PrP for prion diseases, tau and β-amyloid for AD and HD respectively, and α-synuclein for PD. The improper protein conformations are the tertiary structure alterations from α-helix to β-pleated sheets that then favorably follow the aggregation pathways which are thereafter resistant to proteasome degradation pathways [40]. In this regard, even slight modification in the amino acid terminus of proteins means an alteration in the N-degron recognition signaling for degradation [72].

The p38 MAPK phosphorylation pathway has been described as a disease-associated sequela of exposure to the synthetic mRNAs coding for the SARS-CoV-2 spike protein. Moreover, the p38 MAPK phosphorylation pathway inhibits autophagy. This also leads to increased levels of p53. In this way, the formation of the PrP^{SC} infectious isoform triggers a molecular cascade of neurotoxic events that involve the p38 MAPK pathway [60,73].

8. Wip1 Expression and the Resolution of p38 MAPK Activation

Wild-type p53-induced phosphatase 1 (Wip1) is a serine/threonine phosphatase, which plays an essential

role in the resolution of the DNA damage response by downregulating p38-p53 signaling during the recovery phase [74]. Wip1 is overexpressed in many tumors [75,76] and under-expressed in neurons in association with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) [77].

Stressors that induce the p38/MAPK response result in sustained phosphorylation of p53, which not only arrests the cell cycle but can also induce apoptosis when repair processes are overwhelmed with too many DNA double strand breaks. By dephosphorylating several tumor suppressors, most notably p53, Wip1 inhibits apoptosis and promotes tumorigenesis, tumor progression, invasion and metastasis [75].

Tumor cells are often somewhat reckless in proliferating even in the presence of DNA damage, which accelerates their mutation rate, whereas mature neurons are non-proliferating cells even in the absence of stressors. Because the base level of Wip1 is low in neurons, they are more vulnerable to apoptosis following p38/MAPK signaling, because the phosphorylated state is maintained for an extended period of time.

Culmsee and Mattson have commented on the role of p53 in neuronal apoptosis: "p53 production is rapidly increased in neurons in response to a range of insults including DNA damage, oxidative stress, metabolic compromise, and cellular calcium overload" [78]. Their main thesis is that p53 upregulation leads to apoptosis in neurons that eventually results in symptoms of neurodegenerative disease, and they suggested that agents that inhibit p53 may be an effective therapy for neurodegenerative disease [78].

Wip1 expression is controlled through a complicated regulatory process, which begins with p38/MAPK activation. Perhaps surprisingly, Wip1 transcription is upregulated by phosphorylated p53, simultaneous with the upregulation of many tumor suppressor genes, but its translation into protein is delayed. This is because miR-16 is also induced by p53 [79], and this microRNA suppresses translation and promotes clearance of Wip1 RNA [76]. As the repair process progresses, the level of miR-16 falls, such that Wip1 becomes functional only after a delay period, during which the neuron either recovers from the damage or undergoes apoptosis. As more and more neurons die, the symptoms of impaired cognition and memory start to become manifest [78].

9. The Phosphorylation Pathways: Wip1 Expression and the Role of p53.

Experimental data strongly suggest that the p38 MAPK pathway is central to the development of neurodegeneration by infectious prions. The study conducted by C Fang *et al.,* 2018 [13] utilized a specific neuronal culture system that distinguishes the cellular and molecular mechanisms by which prions cause damage in neural synapses. The authors used specific inhibitors against the three main families of MAPK, namely a) the extracellular signal regulated kinases (ERKs), b) the Jun amino-terminal kinases (JNKs), and c) the p38 -stress activated protein kinases (SAPKs) in order to determine which of the distinct subfamilies of kinases are involved in the synaptic toxicity process caused by PrP^{SC}. The authors concluded that the main kinases involved in dendritic spine toxicity were those of p38 MAPK subfamily, and, in particular, the p38α isoform. Furthermore, a p38 MAPK inhibitor, after 24 hours of being added to the culture, was able to completely reverse the initial synaptic toxicity effects caused by PrP^{SC}. Moreover, the authors also used a genetic method of suppressing the p38 MAPK activation cascade by culturing a hippocampal neuron cell line which is heterozygous for p38α MAPK (T180A/Y182F), p38AF. This dominant negative mutant cell line was also protected from PrP^{SC} synaptic neurotoxicity in a way comparable to the effect of the p38α inhibitor. In a relevant study, a double mutation in the activation site of p38AF protein, at the sites of Thr180 and Tyr182, inhibits the phosphorylation of the p38 molecule by other kinases. Also in this study, the heterozygous mice for the p38AF (+/-) allele show a marked reduction in a) p38-related signaling and b) the expression of age-produced cell cycle inhibitors [80].

Additionally, the mutated p38AF animals showed increased proliferation and regeneration of pancreatic islets, amongst other organs. Overall, in this study, the p38AF mutated animals expressing the defective isoform of p38α AF possessed a resistant mechanism that alleviated synaptic toxicity caused by PrP^{SC} (spine degeneration), thereby bypassing the mechanism of PrP^{SC} activation of a localized p38-mediated signaling cascade that leads to dendritic spine retraction [13,80]. Importantly, the p38AF, Wip1 deficient mice showed a reduction in their cellular proliferation capacity. By contrast, the animals that showed Wip1 overexpression

retained their cellular capacity of induced regeneration.

The Wip1 deactivation observed during the natural aging of p38AF mutated animals, concurrent with their genetically induced loss of p38 MAPK activation, is highly relevant to PrP^{SC} propagation by the SARS-CoV-2 spike protein. It shows that spike-protein-induced neurotoxicity, as explained in more detail below, would be predicted to be age-related. The p38 MAPK pathway, being inactivated in the p38AF mutated animals, did not influence the Wip1 activity. Thus, these two distinct but inter-related phosphorylation pathways are being concurrently yet independently inactivated due to aging [80].

Under normal circumstances, the p38 MAPK pathway is activated (phosphorylated) by the upstream induction of Toll-like receptor (TLR) activation via Myeloid Differentiation primary response (MyD88 adapter protein), and downstream by the TGFβ-Activated Kinase 1 (TAK1), which becomes active through autophosphorylation [34]. Moreover, the MyD88 induction involves both TLR2 and TLR4 activation (via the CD14 receptor), with the final outcome being the promotion of the NF-ϰB response [81]. However, it is through the TLR4 activation and subsequent p38 MAPK pathway follow-up of phosphorylation events that the inflammatory response of Il-1β, Il-6 and TNF-α is being presented. The activation of IRAK4 phosphorylation by the SARS-CoV-2 spike protein has been shown to be induced by both TLR2 and TLR4 activation that subsequently produces a similar interleukin-mediated inflammatory response in human macrophages [82]. Furthermore, the same pattern of TLR2 an TLR4 activation to produce NF-ϰB and the interleukin-mediated inflammatory response is also occurring in injured or damaged microglia and astrocytes [83].

Particularly, the TLR4 receptor serves as an upstream regulator of Wip1 phosphatase in cells in the nervous system [84,85]. In astrocytes, Wip1 expression provides a negative feedback loop in response to the activation of the NF-ϰB response. In brief, although TLR4 activation led to an increase in Wip1 and phospho-NF-ϰB-p65 expressions in LPS-stimulated primary astrocytes, the expression of p65 was further increased when the expression of Wip1 was deactivated [84]. Similarly to the LPS-induced activation of TLR4 in human monocytes, the SARS-CoV-2 spike protein induces a comparable interleukin (IL-1β) response also via activating TLR4 [86]. Similar induction of IL-1β was noticed in a differentiated neutrophil cell line that expressed TLR4 following spike protein exposure. Also, the spike protein was able to induce an IL1β response in various murine macrophage cell lines, specifically due to TLR4 expression.

In conditions of brain injury, the expression of Wip1 in the nervous tissue prevents inflammation by inhibiting microglial and macrophage accumulation [87]. In murine and human macrophages, the SARS-CoV-2 spike protein, and specifically the S1 subunit of the trimer, activates NF-ϰB and c-Jun N-terminal kinase (JNK) pathways specifically via TLR4 activation [12]. Additionally, in microglial cells, that are a specialized macrophage type of cell in the brain, the induced spike protein neuroinflammation via TLR4 activation includes sustained NF-ϰB activation, suggesting that Wip1 expression is weak and/or delayed [10]. ROS-dependent activation of JNK causes p53 to robustly induce apoptosis, and this is considered to be a feature in tumor cells, but it may be worrisome when neurons are exposed to JNK activation in the context of highly phosphorylated p53 [88].

Thus, although downregulation of Wip1 expression is positively correlated with a better recovery from sepsis by activating neutrophil migration and thus enhancing antimicrobial activity at the point of infection [89], the loss of Wip1 expression in the nervous system can be viewed as being tightly correlated with increased inflammation by uncontrolled, p65-dependent, induction of NF-ϰB signaling. In that regard, the increased p53 activity can be viewed as a normal function to promote apoptosis in order to prevent the emergence and persistence of cells with damaged genomes [90].

10. Wip1 Activity and Regulation of Expression

Wip1 phosphatase is a critical protein regulating the DNA damage repair processes. Following DNA damage repair, the homeostatic mechanisms of the cell require Wip1 activity to release cells from cell cycle arrest, by dephosphorylating and thus inactivating p53, p38 MAPK, ataxia telangiectasia mutated (ATM) and other stress induced proteins (for review of Wip1 targets, refer to J. Lowe *et al.,* 2013) [91]. With p53 no longer inducing cell cycle arrest, the cell is able to return to its original unphosphorylated state.

The *PPM1D/Wip1* gene was originally discovered as a p53-induced gene. However, it has since been discovered that its expression depends also on many other stress-induced transcription factors apart from p53. Mainly, its product, Wip1, provides a negative feedback loop for the activity of many DNA repair factors including the dephosphorylation, and thus inactivation, of histone 2HX-γ (H2AX-γ), and p53 regulating (inhibitor) molecules [74,92].

Furthermore, the over-expression of Wip1 negatively regulates the NF-κB response by reducing TNF-α induced phosphorylation of the serine 536 of p65 and reducing its binding with p300. The effects of Wip1 activity on inhibition of NF-κB and chromatin remodeling are independent of p38 MAPK pathway activation [93]. Notably, Wip1 expression is decreased when NF-κB activity is inhibited in primary astrocytes, indicating a positive regulation of NF-κB on the *PPM1D* gene and, further on, the neuroinflammatory regulation by Wip1 and NF-κB inhibition [84].

Wip1 expression is reduced during neutrophil activation and is directly inhibited by the increase of microRNA-16 expression which targets its 3' untranslated region and thus regulates post-transcriptionally Wip1 translation. Finally, the TLR4 ligands, and the activation of inflammatory cytokines, downregulate Wip1 expression via the activation of microRNA-16 by p38 MAPK and NF-κB [76,94].

11. Τηε Ρεγυλατιον οφ Ηυμαν Πριον Προτειν ανδ β-Αμψλοιδ Γενες

The *PRNP* gene located in chromosome 20 in humans codes for PrP^{C} in the central nervous system and several other tissues [41]. This is a highly conserved housekeeping gene, and it is subject to many transcription factors functioning in its promoter and thus regulating its expression. Amongst many others putative sequences for transcriptional activation by activator protein 1 (AP-1), SP1 and SP2 (members of the SP/KLF family of transcription factors) have been identified as *PRNP* promoters.

Importantly, a short GC rich region is located upstream from the*PRNP* gene promoter. These GC-rich regions have the potential to form G-quadruplex (G4) structures and therefore regulate gene disease-related expression, as they are subject to favorable binding by p53. Binding of p53 to GC regions forming G4s has been shown to initiate a series of cellular effects related to disease [95,96]. Furthermore, it has been shown that the *PRNP* promoter region harbors a sequence matching the binding sequence of p53. p53 binds directly to the suspected sequence, behaving as a potent PrP^{C} transcriptional activator and enhancer of its mRNA expression [97]. In summary, p53 causes an increased expression of PrP^{C}.

RNA translational regulation is considered an important contributor to PrP^{C} conversion to infectious PrP^{SC}. Beyond DNA, it has been shown that the messenger RNA of PrP^{C} contains five naturally existing consecutive regions forming G4s that are susceptible to G4 binding ligands [98]. In this respect, p53 can be regarded as an RNA chaperone that is able to facilitate the folding of G4s and hence stabilize their structure [99]. G4s in 5'-untranslated mRNA regions are found in multiple neurodegenerative diseases and have been shown to inhibit translation and initiate cap-independent translation [100].

The amyloid precursor protein (*APP*) gene coding for the APP in humans is located on chromosome 21. Viewing its promoter sequence, it can be designated as a housekeeping gene like the *PRNP* gene.*APP* shares some important promoter sequences with *PRNP*like AP-1 and Sp1, amongst many others, which however differ from the sequences in the *PRNP* promoter. This suggests that both genes can be partly transactivated by the activity of common transcription factors [101].

APP mRNA is expressed in a variety of tissues, including muscle, the immune system, and many organs such as the thymus, pancreas, kidneys, the lung and others, in addition to its active expression in the nervous system. However, different variants of APP are cell-type specific in their expression [102].

The variants of APP include APP-like protein-1 (*APPL1* gene located on chromosome 21) and APPL2 (*APPL2* gene located on chromosome 11), which are both type 1 transmembrane proteins with similar structure and topology. Only APP itself, however, contains the Aβ sequence. The fibrillary form of Aβ (40-42 amino acids), found and constituting the primary source of plaques in brains of patients suffering from AD and Down syndrome, originate only from APP proteolysis. The full length of human APP sustains

proteolysis mainly via the α,β,γ-secretases. The derived amino acid sequence of Aβ results from the β-site APP cleaving enzyme 1 (BACE-1) or else β-secretase cleavage yielding APPsβ and APPCTFβ (βAPP) fragments of APP. Thereafter, the cleavage of γ-secretase on βAPP finally yields Aβ and the APP intracellular domain (AIDC) fragments (for details see [103]). Moreover, the AIDC fragment is also produced by α-secretase and subsequent γ-secretase activity.

γ-Secretase is also called presenilin-dependent γ-secretase, since it encompasses presenilin (PS) transmembrane proteins in its catalytic subunit (PS1 or PS2) [104]. In this respect, it has been established that γ-secretase/presenilin-dependent generation of AIDC operates as a transcriptional activator of p53, increasing p53 activity and triggering p53-associated cell death. Moreover, mutations in transcription factor Sp1 increase the p53 activity *in vitro* and in brains of patients affected with familial Alzheimer's disease (FAD) [97]. Mutations on Sp1 are considered as a causative factor for FAD.

The tumor suppressor p53, once generated through the γ-secretase/presenilin dependent transcriptional activation of the *TP53* gene by AIDC bound to Fe65 and Tip60 cofactors, then acts on the promoter of PrP^C and induces the expression of PrP^C mRNA. The p53, γ-secretase/presenilin dependent transactivation of PrP^C expression is abolished in a p53-deficient environment. Thus, it is ultimately the PSs (PS1 or PS2) which exert rate-limiting control over PrP^C expression through their ability to generate AIDC. Finally, βAPP overexpression increases PrP^C expression, whereas βAPP depletion results in lower PrP^C expression, in both *in vitro* and *in vivo* experiments, indicating also the controlling role of BACE-1 activity over PrP^C expression [96]. Thus, the metabolism of APP that produces amyloidogenic products also induces increased production of PrP^C.

12. The Fine Balance between Autophagy and Proteasome Degradation in Relation to Neurodegeneration

A common characteristic of neurodegenerative diseases is a severe disturbance of protein homeostasis. Impaired clearance of misfolded proteins via autophagy/lysosomal degradation results in their accumulation within the cytoplasm [105].

p53 has multi-functional roles in macro-autophagy (hereafter termed as autophagy), a state where the cell suppresses cellular regeneration and consumes/recycles intracellularly its constituents to maintain homeostasis and survival during starvation. Autophagy and p53 exhibit reciprocal functional interactions. p53 operates within a negative feedback loop with the process of autophagy: as p53 activity increases, autophagy is activated within the cell. With increased autophagy, negative feedback suppresses the activity of p53 [106]. During autophagy activation, the intracellular components are delivered to lysosomes for further degradation via both macro- and micro-autophagy pathways, as described in detail by Barbosa *et al.* [107].

The ubiquitin-proteasome system (UPS) and autophagy are two interconnected pathways that mediate the degradation of misfolded proteins. Sequestosome-1, also known as the ubiquitin-binding protein p62, plays a critical role in both pathways. p62 captures and presents ubiquitinated cargos for autophagy [108]. Decreased levels of p62 are linked to many neurodegenerative diseases [109]. Oxidative damage to the p62 promoter decreases p62 promoter activity, reducing expression of p62, and therefore impairing autophagy. Its promoter is particularly rich in guanines that are especially susceptible to oxidative damage [109]. The inhibition of proteasome degradation results in impaired clearing of substrates such as p53 and β-catenin, and this results in a twofold increase in their levels in cellular models. These same elevated levels are reached when the UPS is blocked, even when autophagy is not inhibited.

Since many UPS substrates such as p53 mediate toxicity, impaired removal of such regulatory proteins via autophagy is recognized as a prerequisite for many severe disease states, such as in the case of prion disease, solely due to intracellular increase of aggregation-prone proteins [73]. Furthermore, the activation of autophagic mechanisms is lowered with advancing age, constituting an extra parameter for susceptibility to neurodegenerative disease due to autophagic inhibition [107].

With respect to the development of prion disease, specific *in vitro* and *in vivo* models have shown that

reduced gene expression of p38 MAPK facilitated the clearance of BACE-1 through lysosomal degradation. This resulted in a decrease in the intracellular level and activity of BACE-1, and, ultimately, lower Aβ levels in the mouse brain, associated with enhanced autophagic mechanisms. Thus, knockdown of p38 MAPK in neurons reduces Aβ generation and decreases Aβ load by promoting macroautophagy. Moreover, in a separate experiment, the authors treated human cells with an autophagy inhibitor, and this also increased BACE-1 protein levels, and even abolished the p38-MAPK knockdown-induced decrease of BACE-1 protein. These findings demonstrate that p38 MAPK activation and autophagy inhibition are vital for the progression of prion disease [110].

In relation to SARS CoV-2 spike protein being a toxic factor for prion disease, these findings are of major importance, since infectious prions are shown to activate the p38 MAPK signaling response. In an equal fashion, and in a dose dependent manner, the S1 subunit of the spike protein has been shown to a) increase p38 MAPK protein levels, b) increase phosphorylated p38 levels, c) increase the inflammatory cytokines IL-6 and TNF-α, amongst others, d) increase TLR2/4 protein levels and thus signaling, and e) increase NF-ϰB protein activity and binding to provide transcriptional control over the established neuroinflammation in S1-induced BV2 microglia [13,10].

13. SARS-CoV-2 Spike Protein suppresses DUSPs to Further Induce Neurodegeneration

In addition to Wip1, dual-specificity phosphatases [DUSPs] are a large heterogeneous group of protein phosphatases that can dephosphorylate serine, threonine and tyrosine residues on a large number of proteins. Many of the proteins that they dephosphorylate are part of the MAPK cascade, and therefore they can be effective to turn off MAPK activation and resolve an inflammatory response [111].

Because DUSP genes, especially DUSP1 protein, are negative regulators of p38 MAPK signaling, their reduction under TLR4 signaling will sustain the activation of both p38 MAPK and c-Jun NH2 terminal kinase (JNK) pathways [85,112,113].

As we have seen, several multidisciplinary studies provide evidence of activation of TLR2/4 signaling by the SARS-CoV-2 spike protein [10,11,30,31,86]. Especially in nerve cells, the S1 subunit of the spike protein activates p38 MAPK and NF-ϰB through upregulation of expression and activation of TLR4 pattern recognition receptor [10]. Furthermore, exposure of human macrophages to the spike protein activates the phosphorylation of IRAK4 and the subsequent p38 MAPK and JNK pathways, and resulting suppression of autophagy [82].

Notably, SARS-CoV-2 infection and subsequent cleavage of the spike protein by the transmembrane protease/serine subfamily 2 (TMPRSS2) / p38 MAPK pathway activates MAPK phosphorylation and NF-ϰB signaling by reducing the transcriptional activation of DUSP1 and DUSP5 [114]. This is a unique property of SARS-CoV-2 compared to all other coronaviruses. Moreover, p53 has been shown to enhance the post-transcriptional maturation of miR-16 [79], and, as we have seen, miR-16 has been shown to downregulate expression of Wip1 [76].

Thus, the Wip1 and DUSP inhibitory activity upon p53, p38 MAPK, and ATM will both be attenuated in the presence of the spike protein. As a consequence, there will be sustained production of inflammatory cytokines, and an increased tendency towards cellular senescence and apoptosis [91]. β-Amyloid (Aβ) production occurs in various cell types and in many organs [103,115]. However, in cells orchestrating simultaneous Aβ / AICD production and PrP^C expression, i.e., neurons, the spike-protein-induced impairment of the phosphatase pathways will have deleterious effects, with significant implications for cellular neurotoxicity [116,117].

The excess phosphorylated p53 from the suppression of Wip1 and DUSP dephosphorylation activities acts as a transcriptional activator of the prion protein promoter to produce an excess of PrP^C, creating an environment for prion disease development. Since presenilin-dependent γ-secretase works in concert with p53 by enhancing its expression through producing AICD and Aβ, it thus worsens the preconditioning of spike-protein-induced neurotoxicity in this system. Furthermore, the increased expression of transcription factor AP-1 by the phosphorylated c-Jun triggers the promoters of *APP* and*PRNP* for further transcriptional

activation [97,115].

Common transcription factor activation located in both *APP* and*PRNP* promoters, such as by the selective promoter factor 1 (SP1), happens during the inflammatory response in AD brain. Among many other important roles, AP-1 regulates the transcription of BACE-1, and tau protein subsequently promotes the development of neurotoxicity [118-120]. The condition can be described as 'Wip1 and DUSP deficiency-p53 mediated induction of prion and prion-like disease induced by the SARS CoV-2 spike glycoprotein' and is illustrated in Figure 1.

14. The Relation of SARS-CoV-2 Spike-Protein-Induced Neurotoxicity to Age and the Inhibition of Autophagy

The relationship between age and the reduced cellular capability for autophagy, in combination with p53 accumulation during autophagy inhibition, constitutes the proposed model of spike-protein-induced neurotoxicity presented in Figure 1. In this model, pathogenesis is augmented by a) aging, which leads to impaired autophagy, and b) p53 accumulation, due to the inhibition of the UPS system for degradation [106,107].

Under autophagy inhibition and p38 MAPK activation, a detrimental cascade of events ensues: Wip1 deactivation, and, hence, inhibition of p53 dephosphorylation, concurrent with BACE-1 activation, both promote AIDC positive regulation of the *TP53* gene and the p53-dependent transcriptional activation of the *PRNP* gene. These events set the stage for the cascade of cellular events leading to prion protein aggregation and subsequent pathologies.

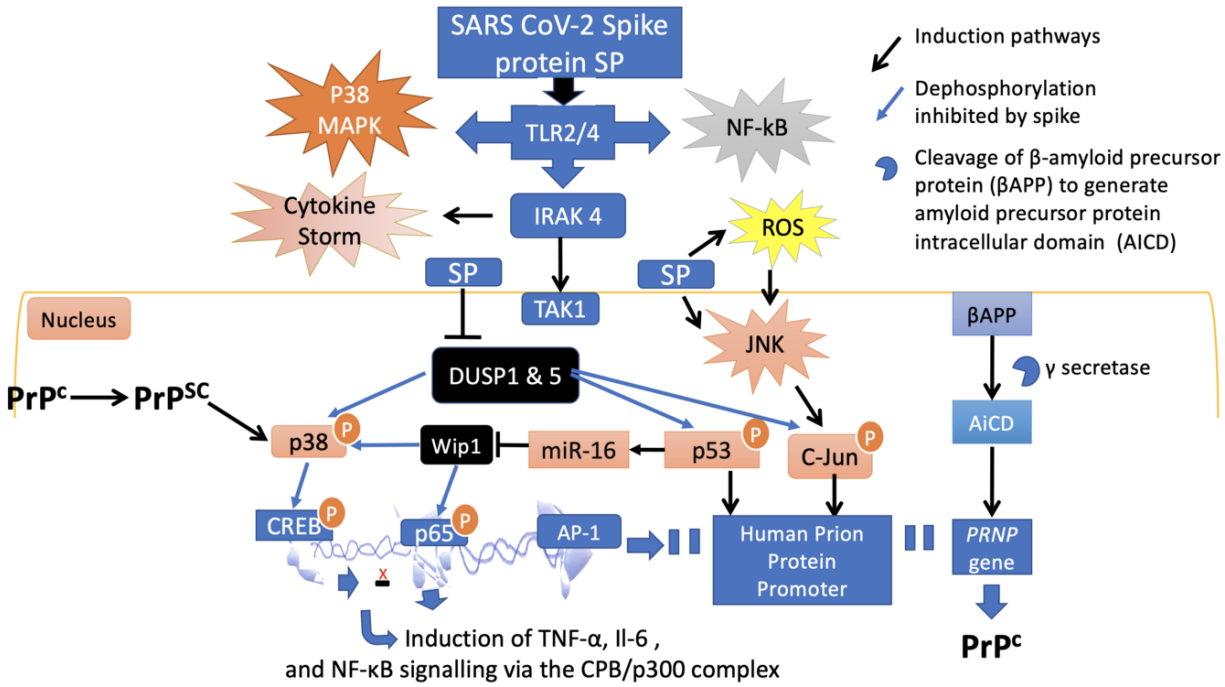


Figure 1:

Figure 1. The phosphorylation pathway induced by SARS-CoV-2 spike protein leads to prion disease. The spike protein activates TLR4 signalling to induce p38 MAPK and NF-ϰB. Moreover, the spike protein also stimulates IRAK4 signalling to induce p38 MAPK, NF-ϰB and cytokine storm and inhibits

DUSPs and Wip1, causing sustained p53 expression. Wip1 deficiency caused by JNK- microRNA-16 activation leads to diminished p53 deactivation and thus, transcriptional activation of the human prion protein promoter. This leads to increased accumulation of PrP^{C} and to induction of IL-6 and TNF-α cytokines through p38/*CREB*, and p65/NF-ϰB activation. Accumulation of PrP^{C} is a predisposing factor for the conformational alteration to PrP^{SC} and therefore prion and prion-like diseases. PrP^{SC}, once formed, will further enhance p38 MAPK activation. Adapted from: [1,10,13,30,84-86,,94,97,112,82,121].

The release of p53 from dephosphorylation by DUSP1 or Wip1 drives the neuron towards the onset of prion and protein folding diseases and establishes the cellular circumstances whereby the SARS-CoV-2 spike protein can play a central role in creating neurotoxicity and predisposing exposed individuals toward neurodegeneration. However, this process is age-dependent, and it is related to the cellular ability to induce autophagy. Although the clear relationship between PrP^{C} and PrP^{SC} formation has not yet been established, the generation of infectious prions is clearly related to the induction of the p38 MAPK pathway, which is also induced by the spike protein in conjunction with JNK in several ways. Figure 2 illustrates the potential mechanisms of the SARS-CoV-2 spike protein, derived either from natural infection or from synthetic mRNAs coding for SP, that induce prion and prion-like disease. The spike-protein-induced neurotoxicity mechanism depends on a) the age of the spike protein recipient and b) the impairment of suppression of prion disease through macro-autophagy [1,13,91,110,82].

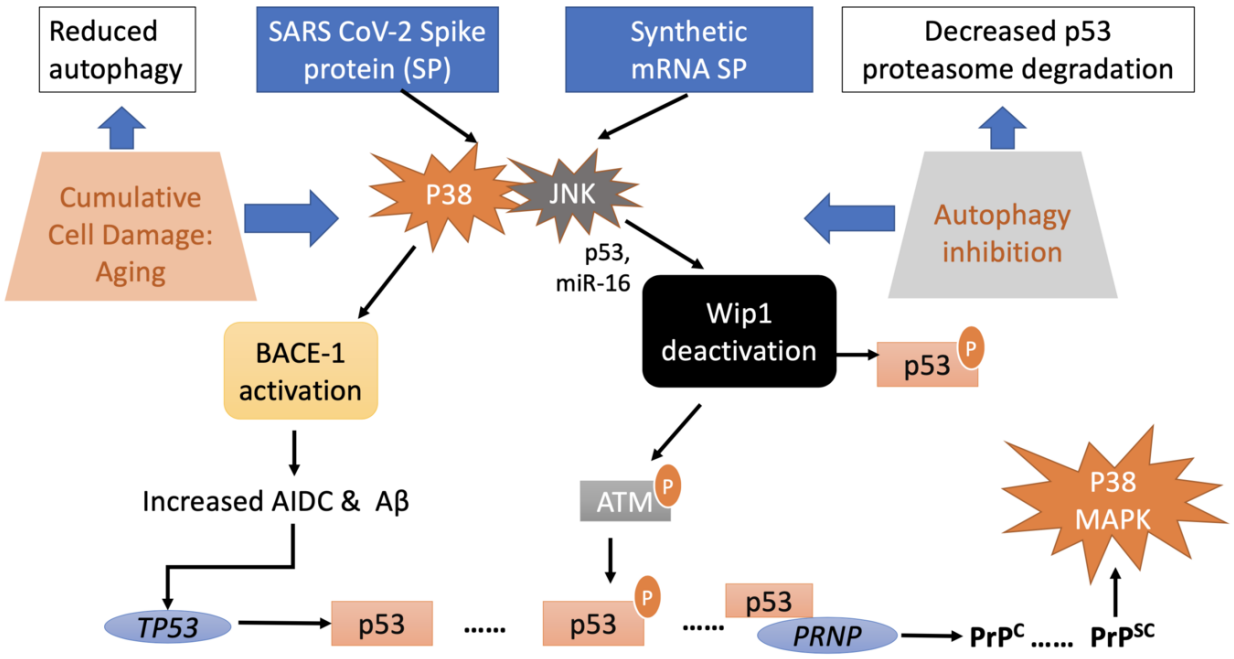


Figure 2. The SARS CoV-2 spike protein neurotoxicity dependence on age and inhibition of autophagy. The ability to induce autophagy is age dependent. Autophagy is inhibited in part through DNA damage to the sequestosome p62 promoter, caused by oxidative stress. The activation of p38 MAPK and JNK pathways by the spike protein in nerve cells leads to BACE-1 activation and, through JNK-mediated Wip1 deactivation, increases activated (phosphorylated) p53. The release of AIDC via APP metabolism further enhances *TP53* transcriptional activation and hence p53 expression. Free P53 can be further phosphorylated by ATM (being active through JNK-dependent microRNA-16 Wip1 inhibition). The overall process leads to accumulation of levels and expression of PrP^{C}. Conformational alteration of PrP^{C}

to PrP^{SC} induces the activation of p38 MAPK, constituting the whole age-dependent process. Adopted from: [10,13,73,91,94,97,106,107,110,112,122].

15. Conclusion

In this paper, we have reviewed the research literature on the spike protein related processes that lead to the development of neurodegenerative disease, in the context of several recent papers reporting on the observed mechanisms of toxicity. We were initially motivated by the observation that COVID-19 patients often suffer from long-term sequelae that include cognitive impairment – so-called long-haul COVID disease. There is also a post-vaccination syndrome that strongly resembles long COVID.

Central to promotion of prion and prion-like disease is the induction of γ-secretase metabolism of the APP sequence, which, through BACE-1, yields the AIDC sequence, a highly potent transcriptional activator of the *TP53* gene. This disease-prone metabolic state is induced through p38 MAPK activation in neurons. Therefore, the SARS-CoV-2 spike protein can be a re-enforcing toxicity factor, since it induces both p38 MAPK and JNK activation which subsequently will provide a surplus of activated p53. The activation of p53 is potentially further enforced through concurrent Wip1 deactivation by JNK-p53-induced miR-16 expression. Decreased degradation of p53 via the UPS and autophagy due to oxidative damage to the p62 promoter further enhances the risk to induction of neuronal apoptosis.

We propose that age-related impairments in autophagy may predispose towards increased risk to cognitive issues associated with the ability of the spike protein to behave as a prion-like protein, triggering misfolding of PrP and other amyloidogenic proteins. The spike protein has been shown to induce an inflammatory response in microglia, which can lead to oxidative stress and DNA damage. Through MAPK activation via TLR4 receptors, as well as JNK activation, the spike protein can be expected to suppress key phosphatases that normally would restore cellular homeostasis following p53 activation via MAPK. Sustained p53 phosphorylation in neurons can induce PrP conversion to PrP^{SC}. The precipitation of misfolded PrP into fibrils causes a loss-of-function pathology, and subsequent catastrophic autophagy failure ultimately leads to programmed cell death (apoptosis) and resulting neurological symptoms and accelerated senescence.

Our work has important implications for public policy given the continued widespread application of COVID-19 vaccines. If the spike protein conceivably could contribute to future neurodegenerative diseases, then the risk-benefit calculation for mass indiscriminate vaccination should be re-examined. If the arguments presented here are found to be true, the vaccinated population has already been subjected to a great deal of harm.

Author Contributions: Conceptualization, A.K., G.N., S.S., and P.M., writing – original draft preparation, A.K., writing - review and editing, A.K., G.N., S.S., and P.M. All authors have read and agreed to the published version of the manuscript.

Funding: Stephanie Seneff was funded in part by Quanta Computers, Taiwan, under the auspices of the Qmulus project. The other three authors received no funding for this work.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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