



## The Mechanistic Target of Rapamycin (mTOR): Novel Considerations as an Antiviral Treatment



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**Abstract:** Multiple viral pathogens can pose a significant health risk to individuals. As a recent example, the  $\beta$ -coronavirus family virion, SARS-CoV-2, has quickly evolved as a pandemic leading to coronavirus disease 2019 (COVID-19) and has been declared by the World Health Organization as a Public Health Emergency of International Concern. To date, no definitive treatment or vaccine application exists for COVID-19. Although new investigations seek to repurpose existing antiviral treatments for COVID-19, innovative treatment strategies not normally considered to have antiviral capabilities may be critical to address this global concern. One such avenue that may prove to be exceedingly fruitful and offer exciting potential as new antiviral therapy involves the mechanistic target of rapamycin (mTOR) and its associated pathways of mTOR Complex 1 (mTORC1), mTOR Complex 2 (mTORC2), and AMP activated protein kinase (AMPK). Recent work has shown that mTOR pathways in conjunction with AMPK may offer valuable targets to control cell injury, oxidative stress, mitochondrial dysfunction, and the onset of hyperinflammation, a significant disability associated with COVID-19. Furthermore, pathways that can activate mTOR may be necessary for anti-hepatitis C activity, reduction of influenza A virus replication, and vital for type-1 interferon responses with influenza vaccination. Yet, important considerations for the development of safe and effective antiviral therapy with mTOR pathways exist. Under some conditions, mTOR can act as a double edge sword and participate in virion replication and virion release from cells. Future work with mTOR as a potential antiviral target is highly warranted and with a greater understanding of this novel pathway, new treatments against several viral pathogens may successfully emerge.

**Keywords:** Akt, angiotensin converting enzyme 2, AMP activated protein kinase (AMPK), apoptosis, autophagy, cytokines, coronaviruses, COVID-19, diabetes mellitus, inflammation, influenza, interferons, interleukins, mechanistic target of rapamycin (mTOR), metformin, mTOR Complex 1 (mTORC1), mTOR Complex 2 (mTORC2), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1- $\alpha$  (MIP-1 $\alpha$ ), oxidative stress, SARS-CoV-2, tumor necrosis factor- $\alpha$ , virion.

### 1. INTRODUCTION

Coronaviruses are viruses that can affect multiple systems throughout the body in both birds and mammals. Coronaviruses belong to the family of *Coronaviridae* and the subfamily of *Orthocoronavirinae*. They are considered to be large ribonucleic acid (RNA) viruses with a genome size ranging from 26 to 32 kilobases, are enveloped viruses, single stranded, and have a positive-sense single stranded RNA with a nucleocapsid of helical symmetry. In some cases, coronaviruses can lead to a non-severe illness with symptoms similar to a mild upper respiratory illness. Yet, in other cases, these viruses that include the  $\beta$ -coronavirus family of Severe Acute Respiratory Syndrome (SARS) virus, Middle East Respiratory Syndrome (MERS) virus, and the COVID-

19 (coronavirus disease 2019) causative agent SARS-CoV-2 can result in severe upper respiratory tract disease [1].

In regards to the disease of COVID-19, the SARS-CoV-2 virus was reported in December of 2019 in Wuhan, China and spread in the population leading to individuals experiencing symptoms of fever, cough, gastrointestinal disturbances, and respiratory dysfunction [2]. The virus has travelled globally with the World Health Organization (WHO) declaring COVID-19 a Public Health Emergency of International Concern, a pandemic, and releasing on-going guidelines to prevent transmission of the virus [3]. To date, SARS-CoV-2 has infected over two million individuals worldwide with the majority of those infected experiencing mild symptoms and recovering usually in fourteen-day period after symptom onset. Yet, a smaller percentage of individuals can become critically ill with multi-system illness and respiratory disease progressing to acute respiratory distress syndrome. Given the global scope of COVID-19, this smaller

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percentage of individuals who are afflicted with debilitating disease can affect both young individuals as well as older individuals [1]. In addition, with efforts to markedly reduce transmission rates, global economies have become challenged, physical and behavior health issues have arisen, and educational hurdles with school closures have developed [4].

## 2. SARS-COV-2 CELLULAR PATHOPHYSIOLOGY AND HYPERIMMUNE RESPONSE

As an infectious virion to humans, SARS-CoV-2 relies upon highly targeted binding to host cells, such as in the nasal epithelial region [5], as well as an excessive and exaggerated activation of the host's immune system [6]. The viral S protein allows SARS-CoV-2 to access host cells through the angiotensin converting enzyme 2 (ACE2) receptor that is expressed in pulmonary, renal, cardiac, vascular, and gastrointestinal cells [1]. During this process, the S protein is cleaved into S1 and S2 with S1 binding to ACE2 and S2 activated by the host surface-associated transmembrane protease serine 2 (TMPRSS2) to allow the virus to fuse with the host cell membrane and become endocytosed by the host cell. Using the host cell's organelles, new viral RNA is translated with necessary structural proteins to ultimately assemble new SARS-CoV-2 virions and infect new cells. In this process, systemic hyperinflammation can occur with the activation and elevation of multiple pro-inflammatory cytokines, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukins, interferons, monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory protein 1- $\alpha$  (MIP-1 $\alpha$ ) [7]. Interestingly, those individuals with significant co-morbidities such as diabetes mellitus (DM) appear to have increased risk for developing significant illness with COVID-19 [8].

## 3. NEW CONSIDERATIONS WITH THE MECHANISTIC TARGET OF RAPAMYCIN (MTOR)

Currently, multiple resources are aggressively being deployed to develop treatments [9] and potential vaccines for COVID-19 [10]. Since therapeutic strategies are mostly symptomatic at present for COVID-19, therapies that can repurpose existing clinically approved antiviral drugs are being considered [11]. Other strategies can include the examination of novel pathways not normally considered to have antiviral treatment capabilities. One such pathway involves the mechanistic target of rapamycin (mTOR) and its associated pathways with mTOR Complex 1 (mTORC1), mTOR Complex 2 (mTORC2), and AMP activated protein kinase (AMPK).

mTOR is a critical pathway that oversees cellular metabolism, development, survival, senescence, tumorigenesis, and inflammation [7, 12-15]. mTOR is a 289-kDa serine/threonine protein kinase. It is also termed the mammalian target of rapamycin and the FK506-binding protein 12-rapamycin complex-associated protein 1 and is encoded by the *FRAP1* gene [16].

mTOR functions as a component of the protein complexes mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) [17-19]. Several strategies can be used to control mTOR activity. As an example, rapamycin is a macrolide antibiotic in *Streptomyces hygroscopicus* that blocks mTOR

activity [20-24]. Rapamycin inhibits the activity of mTORC1 by binding to immunophilin FK-506-binding protein 12 (FKBP12) that attaches to the FKBP12-rapamycin-binding domain (FRB) at the carboxy (C)-terminal of mTOR to interfere with the FRB domain of mTORC1 [20].

## 4. COMPONENTS OF THE MTOR COMPLEXES: MTORC1 AND MTORC2

mTORC1 consists of Raptor, Deptor (DEP domain-containing mTOR interacting protein), the proline rich Akt substrate 40 kDa (PRAS40), and mammalian lethal with Sec13 protein 8, termed mLST8 (mLST8) [18]. mTOR can oversee Raptor activity that can be inhibited by rapamycin [25] as previously described. PRAS40 blocks the activity of mTORC1 by inhibiting the association of p70 ribosomal S6 kinase (p70S6K) and the eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) with Raptor [13, 26-28]. Protein kinase B (Akt) is a part of this pathway since the activity of mTORC1 is increased once Akt phosphorylates PRAS40 [22]. This process releases the binding of PRAS40 to Raptor and sequesters PRAS40 in the cytoplasm with the docking protein 14-3-3 [27, 29, 30]. Deptor prevents the activity of mTORC1 by binding to the FAT domain (FKBP12-rapamycin-associated protein (FRAP), ataxia-telangiectasia (ATM), and the transactivation/transformation domain-associated protein) of mTOR. During the suppression of Deptor activity, Akt, mTORC1, and mTORC2 activities are increased [31]. mLST8 is known to foster mTOR kinase activity. This process involves the binding of p70S6K and 4EBP1 to Raptor [32].

mTORC2 has the components of Rictor, Deptor, mLST8, the mammalian stress-activated protein kinase interacting protein (mSIN1), and the protein observed with Rictor-1 (Protor-1) [33]. mTORC2 regulates cytoskeleton remodeling with PKC $\alpha$  and cell migration with the Rac guanine nucleotide exchange factors P-Rex1 and P-Rex2, and with Rho signaling [34]. mTORC2 can increase the activity of protein kinases including glucocorticoid induced protein kinase 1 (SGK1), a member of the protein kinase A/protein kinase G/protein kinase C (AGC) family of protein kinases. Protor-1 is a Rictor-binding subunit of mTORC2 and increases the activity of SGK1 [35, 36]. mSin1 is needed for the assembly of mTORC2 and for mTORC2 to phosphorylate Akt [37]. Rictor and mSIN1 can enhance cell survival and are known to phosphorylate Akt at serine<sup>473</sup> and promote threonine<sup>308</sup> phosphorylation by phosphoinositide-dependent kinase 1 (PDK1).

## 5. AMP ACTIVATED PROTEIN KINASE

The mTOR pathway is intimately associated with AMPK [38-43]. Together mTOR and AMPK can regulate a number of pathways linked to oxidative stress, mitochondrial dysfunction, and immune system maintenance [13, 15, 43-49]. AMPK can inhibit mTORC1 activity through the hamartin (tuberous sclerosis 1)/tuberin (tuberous sclerosis 2) (TSC1/TSC2) complex that blocks mTORC1 activity [50, 51]. Oversight of the TSC1/TSC2 complex also is controlled through phosphoinositide 3-kinase (PI 3-K), Akt, and its phosphorylation of TSC2. Extracellular signal-regulated kinases (ERKs), glycogen synthase kinase -3 $\beta$  (GSK-3 $\beta$ ),

and protein p90 ribosomal S6 kinase 1 (RSK1) also can regulate the activity of the TSC1/TSC2 complex. TSC2 acts as a GTPase-activating protein (GAP) that converts G protein Rheb (Rheb-GTP) into the inactive GDP-bound form (Rheb-GDP). Once Rheb-GTP becomes active, Rheb-GTP associates with Raptor to control 4EBP1 and mTORC1 binding that increases mTORC1 activity [52]. AMPK phosphorylates TSC2 to increase GAP activity to change Rheb-GTP into the inactive Rheb-GDP and prevent the activity of mTORC1 [53].

## 6. mTOR AND ANTI-INFLAMMATORY CONTROL

Considering that one of the mechanisms that can lead to severe disability and death during infection with SARS-CoV-2 is an exaggerated activation of the host's immune system that results in systemic hyperinflammation with the elevation of multiple pro-inflammatory cytokines, it is interesting to note that mTOR pathways have been tied to immune system modulation [40, 54, 55]. Cytokine interleukin-37 (IL-37) can suppress innate and acquired immune responses. Recently it has been observed that IL-37 during COVID-19 infection is immunosuppressive through mTOR and can increase the activity of AMPK [7]. IL-37 inhibits class II histocompatibility complex (MHC) molecules and inflammation by blocking IL-1 $\beta$ , IL-6, TNF and chemokine (C-C motif) ligand (CCL). As a result, IL-37 with its ability to modulate mTOR and AMPK could be considered as a new target to control hyperinflammation during viral infections that include SARS-CoV-2. In addition, other studies support the premise that AMPK activation can regulate immune system activation. During periods of hyperglycemia, AMPK activation with mTOR inhibition has been shown to prevent cell injury with the reduction of adhesion molecules [41]. However, in some cases activation of mTOR also may play a role in anti-inflammatory mechanisms to limit the toxic effects of reactive oxygen species and maintain mitochondrial membrane potential to prevent cell injury [56]. In studies involving the exposure to oxidative stress, mTORC1 and mTORC2 activation has resulted in the production of the anti-inflammatory cytokine IL-10 to prevent apoptotic cell death [57]. In addition, mTOR activation can block the pro-inflammatory cytokine TNF- $\alpha$  and foster anti-inflammatory cytokine IL-10 activity [58].

Agents, such as metformin, used for the treatment of DM also may be repurposed for potential antiviral activity. Considerations for metformin become relevant since metformin oversees mTOR activity and it has become evident that individuals with co-morbidities with DM have increased risk for developing severe illness with COVID-19 [8]. Metformin relies upon the modulation of mTOR and AMPK to oversee cellular survival [49, 59]. Metformin can block mTOR activity, promote autophagy [60, 61], and may function in an AMPK-independent manner [62]. Yet, additional studies show that metformin can activate AMPK, lead to autophagy induction, and protect against cell apoptosis [63]. Metformin also has been shown to limit lipid peroxidation in the brain and spinal cord and decrease caspase activity during toxic insults that can lead to excessive inflammatory cell activation [49, 64]. Recently, the agent hydroxychloroquine when used as adjuvant therapy with metformin has been shown to

have protective cellular effects in patients with DM by improving glycemic control [65], suggesting that either of these agents may have some role for antiviral treatments. Several clinical studies registered on the United States National Library of Medicine website *ClinicalTrials.gov* are now in preparation or actively recruiting patients to examine both the safety and efficacy of hydroxychloroquine administration in individuals as an antiviral treatment such as for COVID-19.

## 7. mTOR AND ANTIVIRAL ACTIVITY

mTOR has not only been shown to be important for immune system regulation, but studies have shown that mTOR also can regulate viral infection activity. In regards to the West Nile virus, growth of this virion and its expression are dependent upon mTORC1 mediated-regulation of 4EBP/eIF4E interaction and eukaryotic initiation factor 4F (eIF4F) complex formation to support viral growth [66]. Yet, the role that mTOR pathways play with virion growth and expression may vary dependent upon the specific virion. For example, activation of mTOR has been shown to be necessary for anti-hepatitis C activity [67]. Activation of mTOR also has been tied to the ability to promote natural killer cell effector function that can be necessary to destroy virus-infected cells [68]. In relation to influenza A virus, studies illustrate that loss or inhibition of mTOR activity with the corresponding activation of AMPK can promote viral replication [69]. Recent work also suggests that seasonal vaccination for influenza virus in individuals with type 2 DM and treated with metformin, an agent that activates AMPK and blocks mTOR activity, can lead to suppression of type-1 interferon response and ultimately result in limited vaccine effectiveness [70]. However, it is interesting to note that the regulation of mTOR activity may be critical for controlling viral infection and replication. Studies have illustrated that mTORC1 activity can suppress hepatitis C virus replication, but also may foster the packing of the virion and its release from cells [71].

## CONCLUSION AND FUTURE PERSPECTIVES

A number of viral infections in humans can ultimately lead to progressive disease and disability (Table 1). Currently, the SARS-CoV-2 virus has become a global concern. With the infection of more than two million individuals throughout the world, the expansion of COVID-19 among the world's population has led to not only concerns for the development of behavioral health and physical health impairments, but also challenges for global economies. SARS-CoV-2 gains access to host cells through ACE2 receptors and can result in a hyperinflammatory process leading to severe disability and ultimately death.

Given the challenges presented by COVID-19, enormous resources are being used to develop new therapeutic strategies and possible vaccines for the treatment of COVID-19 since current care is primarily symptomatic. A number of investigations seek to repurpose existing antiviral treatments for COVID-19. Yet, other strategies not traditionally considered to have antiviral treatment capabilities may prove exceedingly useful as well. In this respect, mTOR and its asso-

**Table 1. Highlights: mTOR: Novel considerations for antiviral strategies.**

<ul style="list-style-type: none"> <li>• Coronaviruses affect multiple systems throughout the body in both birds and mammals. The <math>\beta</math>-coronavirus family virion, SARS-CoV-2, has quickly evolved as a pandemic leading to coronavirus disease 2019 (COVID-19) highlighting the vital need for innovative strategies to treat viral pathogens.</li> <li>• The mechanistic target of rapamycin (mTOR) and its associated pathways with mTOR Complex 1 (mTORC1), mTOR Complex 2 (mTORC2), and AMP activated protein kinase (AMPK) offer new avenues of opportunity for the development of innovative antiviral treatment strategies.</li> <li>• AMPK activation with mTOR inhibition can limit hyperinflammation and has been shown to inhibit class II histocompatibility complex (MHC) molecules activity, block interleukin1<math>\beta</math> (IL-1<math>\beta</math>), IL-6, tumor necrosis factor (TNF) and chemokine (C-C motif) ligand (CCL) expression, and reduce the presence of adhesion molecules to prevent cell injury.</li> <li>• Individuals with diabetes mellitus have increased risk for developing illness with COVID-19, suggesting that agents such as metformin that promote AMPK activation may have utility to reduce excessive inflammatory cell activation and work with potential adjuvant agents such as hydroxychloroquine.</li> <li>• Yet, it is important to recognize that regulation of mTOR activity may require a precise biological oversight. Activation of mTOR pathways rather than inhibition of mTOR also can offer antiviral activity and be necessary for anti-hepatitis C activity, reduce influenza A virus replication, promote natural killer cell effector function, and be required for type-1 interferon response to raise influenza vaccination effectiveness.</li> <li>• Continued investigations of the mTOR pathway as a potential antiviral target are critical to further unravel the insights required to translate mTOR pathways into clinical strategies that can potentially block viral infection and overcome systemic illness that can be associated with virions such as SARS-CoV-2.</li> </ul>
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ciated pathways with mTORC1, mTORC2, and AMPK offer exciting potential as new antiviral therapies.

Recent studies have shown that mTOR inhibition with AMPK activation may be considered as new targets to control hyperinflammation. AMPK activation with mTOR inhibition can limit class II histocompatibility complex (MHC) molecules activity, block IL-1 $\beta$ , IL-6, TNF and CCL expression, and reduce the presence of adhesion molecules to prevent cell injury. Given that individuals with significant co-morbidities with DM have increased risk for developing illness with COVID-19, addressing avenues linked to metabolic disease and mTOR pathways also may offer some utility. Agents such as metformin that promotes AMPK activation may limit oxidative stress cell injury, reduce excessive inflammatory cell activation, and may be a useful therapy for the treatment of COVID-19 involving potential adjuvant agents such as hydroxychloroquine.

It is important to realize that the development of these antiviral pathways for mTOR is at an early conceptual phase and that other studies support a role for mTOR activation in controlling immune system response and antiviral activity. mTORC1 and mTORC2 activation can promote anti-inflammatory cytokine IL-10 production to block apoptosis. mTOR activation also can inhibit the pro-inflammatory cytokine TNF- $\alpha$  and increase anti-inflammatory cytokine IL-10 activity. Furthermore, mTOR activation has been shown to be necessary for anti-hepatitis C activity, reduce influenza A virus replication, promote natural killer cell effector function, and be required for type-1 interferon response to raise influenza vaccination effectiveness.

## CONCLUSION

Yet, there exists a number of considerations for the mTOR pathway in the development of efficacious antiviral

therapy. Regulation of mTOR activity may require a precise biological oversight. Under some circumstances, pathways of mTOR, such as mTORC1, can suppress hepatitis C virus replication, but also may foster the packing of the virion and the release of the virion from cells. In addition, pathways of mTOR may participate in some virion growth cycles and expression such as with the West Nile virus. In light of the promising early studies with the mTOR pathways as potential antiviral and inflammatory modulatory targets, continued investigations will be critical to gain the necessary insights to translate mTOR pathways into effective clinical antiviral strategies for a number of viral pathogens.

## CONSENT FOR PUBLICATION

Not applicable.

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## CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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