COVID-19 and Liver Cirrhosis: Focus on the Nonclassical Renin-Angiotensin System and Implications for Therapy

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COVID-19 AND LIVER CIRRHOSIS: FOCUS ON THE NON-CLASSICAL RENIN-ANGIOTENSIN SYSTEM AND IMPLICATIONS FOR THERAPY

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List of abbreviations. ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme type 2; ACEi, angiotensin converting enzyme inhibitor; ADAM-17, a disintegrin and metalloproteinase domain-17; Ang II, angiotensin II; Ang1-7, angiotensin 1-7; ARB, angiotensin type 1 receptor blocker; AT_1R, angiotensin type 1 receptor; des-Arg1-BK, des-arginine bradykinin; ERK, extracellular signal-regulated kinase; ET-1, endothelin-1; HSC, hepatic stellate cells; MasR, Mas receptor; RAS, renin-angiotensin system; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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ABSTRACT

Angiotensin-converting enzyme type 2 (ACE2) is the cell receptor of SARS-CoV-2, the viral agent of COVID-19. ACE2 and a network of further enzymes and receptors constitute the non-classical renin-angiotensin system. ACE2 cleaves angiotensin II, which promotes vasoconstriction, oxidative stress, liver and lung inflammation and fibrosis, into angiotensin 1-7 (Ang1-7), which binds to Mas receptors (MasR), resulting in arterial vasodilatation, natriuresis, anti-inflammatory and anti-fibrotic effects in tissues. Viral binding to ACE2 allows viral entry into human cells including hepatocytes, followed by viral replication and host cell depletion of ACE2. The coronavirus-dependent demise of ACE2 and its product (Ang1-7) leads to cytokine activation and cytokine-induced hepatocyte apoptosis and necrosis, which in turn decreases liver reserve and may induce hepatic injury. Approximately one third of patients with cirrhosis, especially those with decompensation, die after a median of 10 days from COVID-19 diagnosis, and nearly two-thirds of these deaths occur before intensive care unit admission for COVID-19-related pulmonary insufficiency. In these cases, liver function deteriorates rapidly after hospital admission, suggesting that cirrhotic patients frequently die from accelerated end-stage liver disease. Pharmaceutical interventions which may provide novel strategies to counter liver cirrhosis decompensation due to COVID-19 include non-peptidic MasR agonist AVE0991, which replaces the anti-inflammatory and anti-fibrotic effects of Ang1-7, and metallopeptidase neprilysin inhibitor candoxatrilat, which reduces Ang1-7 clearance and causes portal pressure reduction with increased natriuresis in experimental cirrhosis. Moreover, SF2809E, an inhibitor of serine protease chymase (an enzyme generating most tissue angiotensin II) may also block TMPRRS2, a host serine protease that primes SARS-CoV-2 spike glycoprotein before adhesion to ACE2. These and further drugs deserve consideration in patients with COVID-19 and hepatic comorbidities.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive-sense single-stranded RNA virus, and the syndrome it causes is named **CO**rona**VI**rus **D**isease outbreak or COVID-19, which was first diagnosed in the year 20**19**. To date, more than one and a half million patients have died from the illness worldwide.

In severe clinical cases, SARS-CoV-2 infects type II pneumocytes and leads to acute respiratory distress syndrome. Comorbidities, including chronic liver diseases, arterial hypertension, obesity, diabetes, cancer, cardiovascular and pulmonary dysfunctions, worsen the prognosis of patients with COVID-19 (1). This is because the cell membrane-bound metallopeptidase angiotensin-converting enzyme type 2 (ACE2), the primary entry receptor for SARS-CoV-2, is ubiquitous, leading to multiple organs being involved in COVID-19 (2, 3). ACE2, the viral receptor, is also an integral part of the non-classical or tissue renin-angiotensin system (RAS).

The non-classical RAS (Figure 1)

The non-classical RAS is a network of enzymes and angiotensins, derived from angiotensin II (Ang II) and involved in the regulation of inflammation and tissue blood perfusion. The major player is ACE2, a transmembrane protein with an extracellular N-terminal domain containing a mono-carboxypeptidase site and a transmembrane C-terminal tail. ACE2 N-terminal domain is the SARS-CoV-2 binding site (2, 3). ACE2 cleaves the Pro_7-Phe_8 bond of Ang II to form angiotensin 1-7 (Ang1-7), which binds to cell membrane G protein-coupled receptors called Mas receptors (MasRs). This leads to vasodilatation and increased natriuresis through the production of arachidonic acid and increased cell levels of cyclic GMP (4).

The non-classical RAS extends well beyond the ACE2-Ang1-7-MasR axis. Ang1-7 can be transformed into heptapeptide alamandine by an aspartate decarboxylase that converts Asp_1 of Ang1-7 into Ala_1 of alamandine, which binds to Mas-related G protein-coupled receptor member D, leading to arterial vasodilatation (5). Cellular Zn-metallo-endopeptidase neprilysin can generate Ang1-7 from angiotensin I but continues to metabolize Ang1-7 at the Tyr_4-Ile_5 bond to form inactive byproduct angiotensin 1-4. Moreover, serine endopeptidase chymase, present in liver, heart, kidney and ubiquitous mast cells, converts angiotensin I into Ang II as angiotensin-converting enzyme (ACE) does in the endothelium (4).

The RAS inside the liver

ACE, Ang II and its angiotensin type-1 receptor (AT_1R), as well as ACE2, Ang1-7, MasRs, chymase and neprilysin are expressed in the healthy and, especially, in the diseased liver, in which they modulate vascular tone and the development of progressive fibrosis during chronic injury. In the diseased liver, hepatic levels of Ang II are already elevated in pre-cirrhotic animal models of liver fibrosis (6). Whilst the endothelium produces Ang II through ACE, chymase produces 80% of Ang II found in tissues. Ang II leads to hepatic stellate cell (HSC) activation and production of pro-fibrotic cytokines and connective tissue growth factors. Activated HSCs themselves express *de novo* ACE and AT_1Rs and also synthesize Ang II (6). Ang II also activates infiltrating inflammatory cells, leading to their proliferation, production of inflammatory mediators (interleukin-6 and tumor necrosis factor-α) and chemotaxis. Therefore, the RAS system is already active inside the liver before the onset of cirrhosis. In patients with decompensated cirrhosis, hepatic levels of Ang II are much more elevated, spilling into the systemic circulation. This is related to chymase being massively upregulated in hepatocytes of regenerative nodules and α-smooth muscle-positive HSCs (7). In tissues with chronic inflammation, chymase also converts big endothelin-1 (ET-1) into profibrotic polypeptide ET-1 (7).

Liver neprilysin, another enzyme in the system, causes Ang1-7 degradation and ET-1 production from big ET-1 and ET_{1-31}. In the cytosol fraction of the cirrhotic rat liver, neprilysin content is increased by 280%. This enzyme is localized mostly in desmin-positive activated HSCs of fibrotic septa (8).

ACE2 expression and Ang1-7 synthesis are also increased in the cirrhotic liver (9), and the latter peptide is a negative regulator of the classical ACE-Ang II-AT_1R axis. Ang1-7 binds to the C-terminal domain of ACE and reduces Ang II generation (10). Experimental liver fibrosis is aggravated by MasR antagonists (6) and relieved by recombinant ACE2 (11). Several studies have shown that activated ACE2-Ang1-7-MasR axis reduces release of proinflammatory cytokines (tumor necrosis factor-α, interleukin-6 and transforming growth factor-β) that may cause liver cell apoptosis and necrosis followed by tissue fibrosis (10). Thus, it appears that having excess ACE2 is advantageous to the patient with liver cirrhosis.

In the cirrhotic liver, due to ACE, chymase and neprilysin overexpression, faced by the sole increase in ACE2 activity, some five-fold increase in the Ang II/Ang1-7 ratio occurs, compared to normal liver (4). Any further damage to hepatic ACE2-Ang1-7-MasR axis may lead to end-stage liver disease. This occurs during COVID-19 clinical course.

SARS-CoV-2 and the Liver

ACE2 protein (SARS-CoV-2 receptor) is detectable at low levels in healthy livers, mostly in endothelial cells, occasional bile duct cells, and sparse centrilobular hepatocytes (9). In human cirrhosis, liver ACE2 mRNA levels are upregulated 34-fold, ACE2 protein 97-fold, and ACE2 immunostaining is identified in 80% of hepatocytes (9). Therefore, cirrhotic liver is highly susceptible to SARS-CoV-2 infection. Overexpression of ACE2 and increased synthesis of Ang1-7 are also found in the mesenteric arterioles of patients with cirrhosis, contributing to the mesenteric hyperdynamic circulation that supports portal hypertension (12, 13). After viral entry, SARS-CoV-2 RNA is found in the liver, along with microvacuolar steatosis, syncytial multinuclear hepatocytes, lobular and portal inflammatory activity (14). In fatal cases, platelet-fibrin microthrombi are identified in hepatic sinusoids at post-mortem, and this is due to SARS-CoV-2-dependent endothelial dysfunction and coagulopathy (1). Transaminase levels are increased in up to 62% of patients with severe COVID-19. Infected patients show hypoalbuminemia and elevation of gamma-glutamyl transferase, but significant cholestasis is rare (15). In patients with underlying hepatic steatosis, >50% of COVID-19 cases are severe, with 17% mortality. Liver cirrhosis is an independent risk factor of mortality in COVID-19: the combined COVID-HEP and SECURE-Cirrhosis registries confirm that 38% of cirrhotic patients with COVID-19 had worsening ascites, encephalopathy, or acute kidney injury, and 40% of them died (16). In a group of cirrhotic patients with COVID-19 from North America, mortality was 30%, while that in patients with COVID-19 alone was 13% (17). Among 50 Italian cirrhotic patients with COVID-19 (18), 30-day mortality was 34%; 64% of patients needed non-invasive respiratory support; when compared to the last outpatient visit before SARS-CoV-2 infection, bilirubin, INR, ALT and creatinine significantly increased, whilst albumin levels decreased; the percentage of patients with Child-Pugh score C rose from 12% to 33%. Other contributors to increased mortality in cirrhosis during this pandemic include delayed screening or cancellation of elective therapeutic procedures for varices and hepatocellular carcinoma. Acute liver injuries related to drugs used to treat COVID-19 have been described (15).

SARS-CoV-2 mechanisms of liver damage (Figures 2 and 3)

The spikes of SARS-CoV-2 envelope have a S glycoprotein that contains two functional domains: an S1 ACE2-binding domain and an S2 domain essential for fusion of viral envelope and cell membranes (19). Cellular trypsin-like serine protease TMPRSS2 cuts between S1 and S2 to allow viral entry into cells. TMPRSS2 is highly expressed in hepatocytes and priming of viral spikes is critical for infection (19). Host furin, an enzyme of the subtilisin-like proprotein convertase family (3), and plasmin, a furin-like protease (20), may also prime SARS-CoV-2 S glycoprotein, at a site different from TMPRSS2 cleavage site. Once S glycoprotein is primed, clathrin-dependent endocytosis of the parent virions into cells occurs (19).

High plasmin levels are found in cirrhotic patients, related to increased tissue-type plasminogen-activator activity and decreased alpha 2-antiplasmin (21). Targeting plasmin with inhibitors (aprotinin, tranexamic acid) may be an option to reduce hyper-fibrinolysis (critical to disease progression in COVID-19) and viral spike priming in infected cirrhotic patients (20).

SARS-CoV-2 infection prompts inflammatory responses that depend on cellular depletion of ACE2 (19). Firstly, internalization of ACE2 bound to virions reduces its availability on the cell surface. Secondly, unknown viral mediators induce gene expression of a disintegrin and metalloproteinase domain-17 (ADAM-17). ADAM-17 functions as a "sheddase", releasing anchored ACE2, interleukin-4 and interferon γ (IFNγ) from the membranes of cells, including hepatocytes. Finally, free interleukin-4 and IFNγ further downregulate membrane-bound ACE2. TMPRSS2, beyond priming SARS-CoV-2 spikes, also cleaves ACE2 and competes with ADAM-17 for ACE2 extracellular shedding (19). Loss of ACE2 leads to shift of the RAS to higher Ang II and lower Ang1-7 tone. In COVID-19 patients, plasma Ang II levels increase and this correlate with the viral load in bronchoalveolar lavage fluid (1).

Moreover, ACE2 annihilation by SARS-CoV-2 means that clearance of inflammatory mediator des-arginine bradykinin (des-Arg1-BK) is reduced during infection, because ACE2 metabolizes this peptide, which promotes pulmonary inflammation in COVID-19 via stimulation of bradykinin B1 receptors in lung endothelial cells (22). Overexpression of endothelial bradykinin B1 receptors

occurs in livers of rats with experimental cirrhosis (23). This may further fuel inflammation in the cirrhotic liver.

Ang II, through activation of extracellular signal-regulated kinase (ERK)1/ERK2, further reduces ACE2 cell expression (24). Therefore, any compound that blocks Ang II function, such as ACE inhibitors (ACEis) or AT_1R blockers (ARBs), should increase ACE2 activity, as repeatedly shown in several experimental settings (2). The conundrum is that ACEis and ARBs may improve outcomes in patients with COVID-19 through reduced Ang II function, but both drug classes increase ACE2 expression and potential susceptibility to SARS-CoV-2 infection (2). Recent meta-analyses demonstrate that RAS inhibitors might be associated with better prognosis in hypertensive patients with COVID-19 (25) or, at least, that ARBs or ACEis should be continued in these subjects (26), as ACEis also reduce Ang1-7 clearance (Figure 1). However, in patients with ascitic cirrhosis, ACEis and ARBs are not recommended because they significantly increase the risk of hypotension and renal failure (4). Unfortunately, this also holds true in decompensated cirrhosis with COVID-19.

Potential therapeutic options (Table 1)

Drugs in development affect the non-classical RAS and may be beneficial to cirrhotic patients with COVID-19. The principles whereby these drugs could help are 1) reduced viral entry and 2) increased Ang1-7 activity.

Metallopeptidase inhibitor MLN-4760 causes a conformational change in the extracellular domain of ACE2 that mimics the closing of a clam shell, thereby potentially reducing SARS-CoV-2 cell binding and viral spread to vital organs (27). In isolated and perfused bile-duct-ligated liver of rat, intraportal MLN-4760 infusion increases Ang1-7 production, an effect abolished by neprilysin inhibition, showing that, when MLN-4760 interferes with ACE2, liver neprilysin mostly converts angiotensin I into Ang1-7, rather than degrading the latter (28) (Figure 1). This hepatic upsurge of Ang1-7 could prove protective in cirrhotic patients with COVID-19. Since mesenteric ACE2 also contributes to the splanchnic hyperdynamic circulation of cirrhosis (12, 13), MLN-4760 is expected to reduce portal pressure in cirrhotic patients with COVID-19 (28).

In COVID-19 patients, replacement of Ang1-7 function is helpful. Compound AVE0991 is a nonpeptidic Ang1-7 analog. AVE0991 can be administered orally and reduces intrahepatic resistance to portal flow and portal pressure in rats with experimental cirrhosis (29).

Reduced Ang1-7 clearance would benefit COVID-19 patients. Candoxatrilat inhibits endopeptidase neprilysin, which cleaves Ang1-7 into angiotensin 1-4, and therefore increases Ang1-7 hepatic concentrations. In experimental liver cirrhosis, candoxatrilat acutely reduces portal pressure and increases solute-free water clearance and urinary excretions of sodium and cyclic GMP, without effects on arterial pressure and plasma renin activity (8, 30).

Trypsin-like serine protease inhibitors (camostat and nafamostat mesylates) may prevent host TMPRSS2 from priming viral spike glycoprotein (31). Trypsin-like serine protease inhibitor SF2809E blocks chymase, the main source of Ang II in tissues, and might prevent TMPRSS2 from priming the spike glycoprotein. SF2809E decreases liver and kidney Ang II content, reduces portal pressure and liver fibrogenesis, and increases natriuresis in experimental CCl_4 cirrhosis (7).

Soluble ACE2 converts Ang II into Ang1-7 in extracellular fluids and antagonizes SARS-CoV-2 binding to transmembrane ACE2 (Figure 3). Recently, in early COVID-19, recombinant human ACE2 has been delivered intranasally, avoiding the arterial hypotension observed during ACE2 intravenous infusion (31).

Conclusions

Hepatic overexpression of SARS-CoV-2 receptor (ACE2), ACE and bradykinin B1 receptors, along with systemic hyperplasminemia, predispose cirrhotic patients to a severe COVID-19 clinical course. In order to modify SARS-CoV-2 interaction with ACE2, mimic beneficial Ang1-7 effects, prolong Ang1-7 half-life, or reduce tissue production of Ang II, suitable drugs exist, although still not available on the market. These drugs deserve further development in the context of liver cirrhosis with COVID-19.

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Figure legends

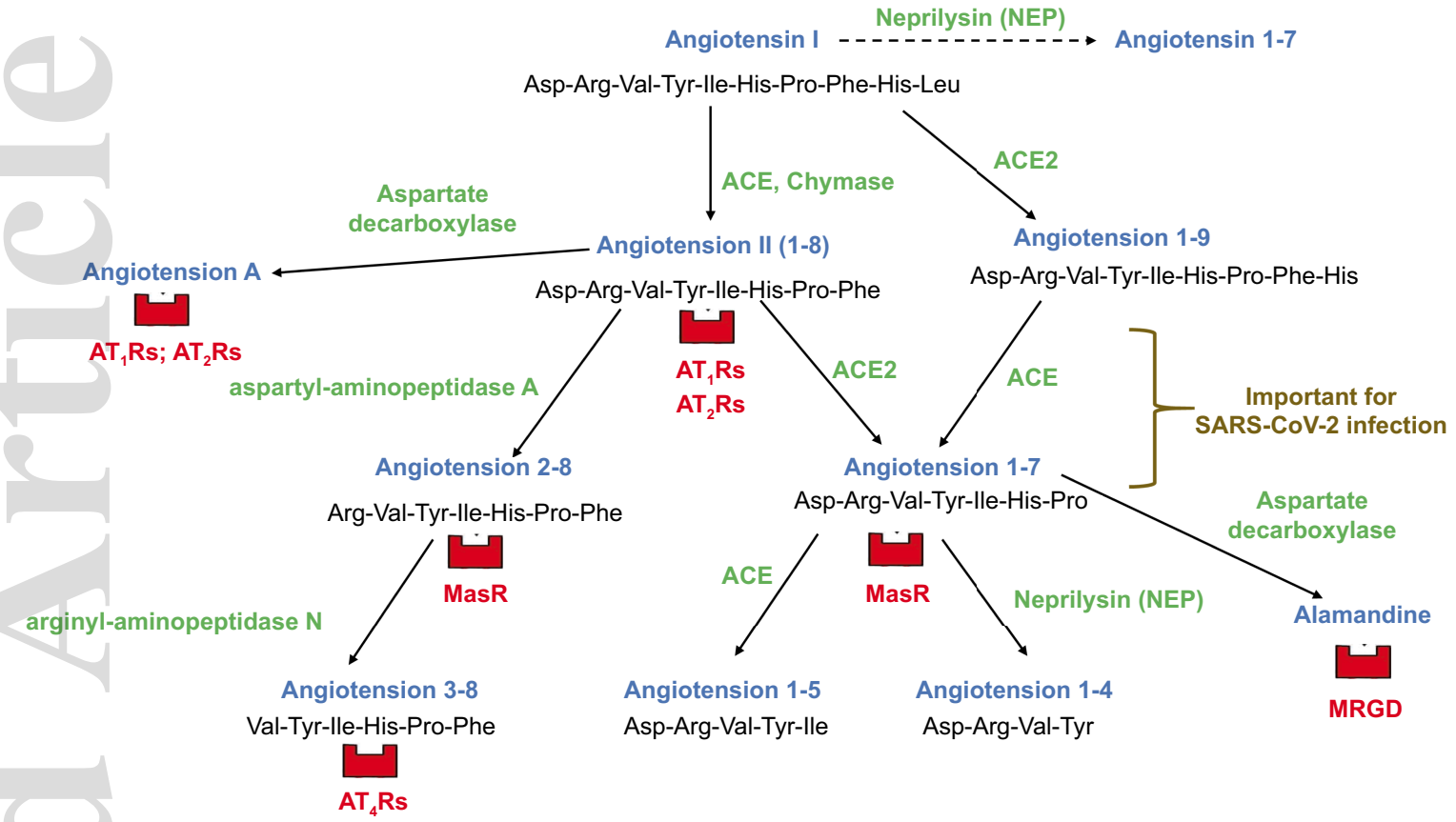
Figure 1. Diagram depicting pathways of synthesis and degradation of angiotensins in classical and non-classical RAS, with respective receptors for each bioactive peptide. Classical RAS is defined as the ACE-Ang II-AT_1R axis; the non-classical RAS is composed of the ACE2-Ang1-7-MasR axis, further metabolism of Ang1-7, and the Angiotensin 2-8/Angiotensin 3-8 pathway. The main degradative pathway of Ang II in humans is through the sequential actions of plasma aminopeptidases A and N. ACE2: angiotensin-converting enzyme type 2; $AT_{1\text{-}2\text{-}4}Rs$: angiotensin type 1-2-4 receptors; MasR: Mas receptor; MRGD: Mas-related G protein-coupled receptor member D.

Figure 2. Mechanisms of Covid-19 binding to cell membrane ACE2, viral spike glycoprotein priming into S1 and S2 subunits by host proteases furin, plasmin and TMPRRS2, and viral entry along with ACE2 into human cells. Protease furin may be found as free-floating in the extracellular fluids or as membrane-bound enzyme. N-terminal domain of ACE2 is the extracellular viral binding site, while ACE2 C-terminal tail anchors the enzyme to plasma membranes.

Figure 3. SARS-CoV-2 causes ACE2 cellular depletion through ADAM-17 upregulation, and ADAM-17-mediated IL-4 and IFNγ cellular shedding into extracellular fluids. In turn, free IL-4 and IFNγ further downregulate ACE2 cellular expression. Arginine and lysine residues within ACE2 amino acids 697 to 716 are essential for ACE2 cleavage by TMPRSS2; ADAM-17 requires arginine and lysine residues within ACE2 amino acids 652 to 659 for cleavage. Right hand side of the picture: C-terminal ACE2 fragments of 13 kDa results from TMPRSS2 processing of cellular ACE2. Left hand side of the picture: soluble ACE2 (sACE2), obtained through action of ADAM-17 on cellular ACE2, is the complete N-terminal ectodomain of the enzyme, still able to bind SARS-CoV2 and convert Ang II into Ang1-7 in the extracellular space.

Table 1. Drugs affecting non-classical RAS and COVID-19.

~~**Comorbidities, drugs**~~ **Drugs influencing non-classical RAS**	**Biomolecular and functional mechanisms**	**Pathophysiological consequences in COVID-19**	**Potential advantages in COVID-19**
Angiotensin-converting enzyme inhibitors (ACEis).	Increase cellular ACE2 expression, decrease Ang 1-7 clearance	Increase cellular adhesion of SARS-CoV-2.	Less inflammatory damage due to increased ACE2.
Angiotensin receptor blockers (ARBs)	Increase cellular ACE2 expression.	Increase cellular adhesion of SARS-CoV-2.	Less inflammatory damage due to increased ACE2.
Metallopeptidase inhibitors (e.g. MLN-4760)	Conformational change of ACE2	Decrease cellular adhesion of SARS-CoV-2	Limited viral spread to vital organs
Nonpeptidic Ang1-7 analogs (e.g. AVE0991)	Agonist effects on MasRs	Replacement of reduced Ang1-7 function	Less inflammatory damage
Neprilysin inhibitors (e.g. candoxatrilat)	Decrease Ang1-7 clearance	Prolongation of Ang1-7 half-life	Less inflammatory damage
Trypsin-like serine protease chymase inhibitors (e.g. SF2809E)	Reduce Ang II local release; inhibition of SARS-CoV-2 spike glycoprotein priming	Decrease cellular adhesion of SARS-CoV-2	Less inflammatory damage; limited viral spread to vital organs



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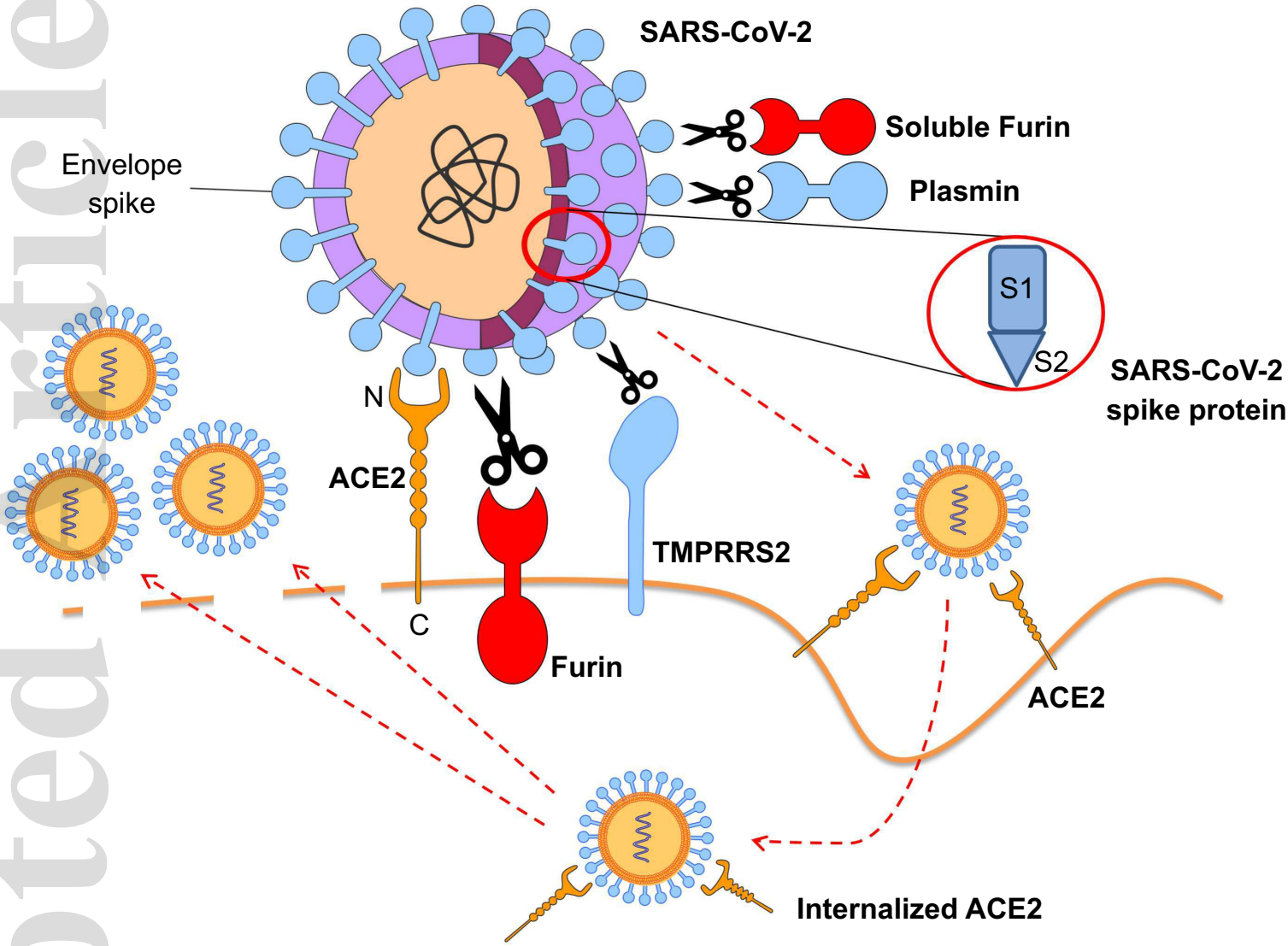

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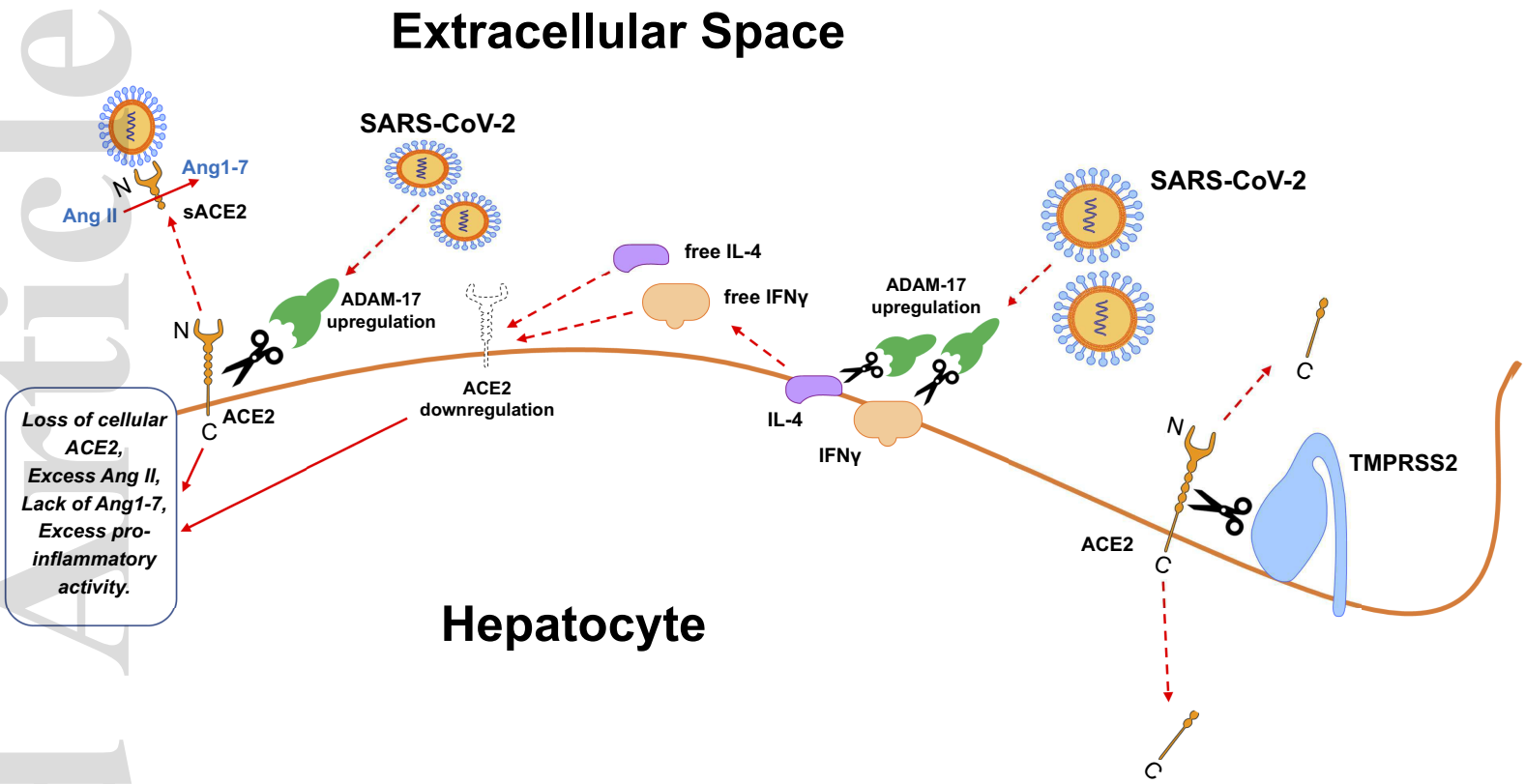

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