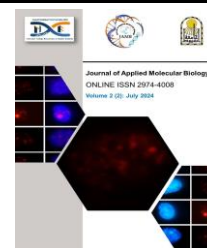


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<https://jamb.journals.ekb.eg>**Review****Emergence and Characteristics of the SARS-CoV-2 Outbreak: From Identification to Cellular Entry Mechanisms**Dalia Zanati¹, Omnia EL Badawy², Manal Elkhwaga³ and Mohamed A El-Mokhtar^{2,*}¹Molecular Biology and Research Studies Institute, Assiut University, 71516 Assiut, Egypt²Medical Microbiology and Immunology Department, Faculty of Medicine, Assiut University, 71516 Assiut, Egypt³Chest Department, Faculty of Medicine, Assiut University, 71516 Assiut, Egypt*Corresponding Author: elmokhtar@aun.edu.eg**ARTICLE INFO****Article History:**

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ABSTRACT

Several Wuhan hospitals received clusters of patients with pneumonia in late December 2019; the cause of these cases, at that time, remained unknown and on January 30, the WHO recognized the ongoing coronavirus outbreak as a global public health emergency. The International Committee on Taxonomy of Viruses officially named the new coronavirus "SARS-CoV-2" on February 11, whereas the WHO classified the disease as "COVID-19". New research shows that SARS-CoV-2 uses the ACE2 receptor to access cells, working in tandem with the host's Tmprss2. Research has discovered that cells that have a greater amount of ACE2 expression are more vulnerable to the SARS-CoV S protein. This implies that higher levels of ACE2 could heighten the likelihood of SARS-CoV-2 infection. The aim of the article could be to provide a comprehensive overview of the emergence and key characteristics of the SARS-CoV-2 outbreak, tracing its origins and spread. Additionally, the molecular pathways involved in cellular entry was discussed. In addition, the implications for both public health and scientific research were addressed.

INTRODUCTION

Infectious coronaviruses can cause respiratory diseases in humans and many other species. The public's concern over the spread of these viruses peaked between 2002 and 2012 due to the appearance of particularly dangerous examples, including SARS-CoV and MERS-CoV [1]. Genomic structure and evolutionary links provide credence to their close association. Although most coronaviruses affect birds, some can infect mammals as well [2]. Key stages in the infection process of SARS-CoV-2 in host cells involve the use of Angiotensin-Converting Enzyme 2 (ACE2) and Transmembrane Protease Serine 2. Soluble ACE2 (sACE2) reduces blood pressure during the Renin-Angiotensin-

Aldosterone System protection phase by interacting with the Mas receptor and hydrolyzing the methyl terminal amino acid phenylalanine [3, 4].

SARS-CoV-2 and COVID-19

A varied class of respiratory viruses known as coronaviruses can infect a large variety of animals and humans. The symptoms might vary in intensity, ranging from moderate to severe. In 2002, a zoonotic coronavirus called severe acute respiratory syndrome (SARS-CoV) was detected in animals. In 2012, the other zoonotic coronavirus, Middle East respiratory illness (MERS-CoV), was transmitted to humans. Within this particular framework, novel coronaviruses provide a significant peril to the well-being of the general population. In late 2019, a novel coronavirus named SARS-CoV-2 produced an outbreak of atypical viral pneumonia in Wuhan, China [5]. The virus was highly contagious and infected a wide variety of humans. The severity of the symptoms could be mild, moderate, or severe [6].

According to virus taxonomists, coronaviruses belong to the subfamily and order Nidovirales. The family Coronaviridae includes the Coronavirinae. There are four identified species in the subfamily: delta, gamma, beta, and coronaviruses [7]. Considering the connections between genomic architecture and evolution, birds are the primary reservoirs for gamma and delta coronaviruses, however, many mammals are not immune [8]. Coronaviruses, both alpha and beta, induce respiratory diseases in humans and gastroenteritis in animals. In humans, SARS-CoV and MERS-CoV respiratory infections are catastrophic, whereas in healthy people, HCoV-NL63, HCoV-229E, HCoV-OC43, and HKU1 are known to cause mild upper RTI [9].

Certain alpha and beta coronaviruses can cause severe illness in cattle. Moreover, Porcupine transmissible gastroenteritis virus, porcine enteric diarrhea virus, and swine acute diarrhea syndrome coronavirus can affect pigs [10].

Sequencing databases show that all coronaviruses that infect humans have their origins in birds. Prior to this, it was believed that rodents were the most likely hosts for HCoV-OC43 and HKU1, whereas bats were the initial hosts for SARS-CoV, MERS-CoV, HCoV-NL63, and HCoV-229E. Domestic animals play a vital role as middlemen in the transmission of viruses from their original hosts to humans [11].

The virus consists of a round 100-160 nm capsid that encloses a positive-sense ssRNA genomes that range in size from 27 to 32 kilobases (kb). The first two-thirds of the genome at the 5' end are made up of sixteen non-structural proteins, including the polyprotein pp1ab, which are responsible for transcription and genome replication. Numerous structural proteins, including envelope glycoprotein spike (S), nucleocapsid (N), and membrane (M), are encoded by the 3' end [12]. Some viral genes are essential for replication, while others code for structural proteins and are species specific [13].

Bats' ability to recombine with other coronaviruses allowed the new coronavirus SARS-CoV to emerge. Humans and civets were infected with a recombinant virus in the years before the SARS outbreak. The MERS-CoV that infects camels was most likely transmitted by bats at least thirty years ago. When immune-competent people are affected, HCoV-229E and HCoV-NL63 usually produce moderate illnesses. The most likely source of HCoV-229E, according to recent research, may have been camelids, an intermediate host between humans and African bats [14]. According to recent research, HCoV-229E may have migrated from African bats to camelids and other mammals [15, 16].

TMPRSS2 (transmembrane serine protease 2)

Several viruses infect humans using the protease TMPRSS2, including SARS-CoV-2. Additionally, it is utilized by carcinogenesis, particularly in the development of androgen-responsive prostate cancer. The initial stage in the life cycle of this 70 kDa serine protease involves autoproteolytic activation [3].

Similar to humans, animals lacking TMPRSS2 or with a mutated version of the gene do not exhibit any symptoms. This study provides evidence that type II transmembrane serine proteases (TMPRSS2 and its related proteins) engage in shared activities throughout the family of proteases. One possible explanation is that the protein's specialized, non-essential function becomes obvious in reaction to stress, disease, or other systemic problems [17].

Normal function of TMRSS2:

The TMPRSS2 protein plays important role in different physiological and pathological processes, such as digestion, shape, blood clotting, reproduction, inflammation, invasion of malignant cells, cell death, and pain. The level of TMPRSS2 is controlled during development and rises with age [18, 19].

Expression of TMPRSS2:

An adult's expression level is lower than that of a growing fetus's brain. Compared to mature male and female lung tissue, fetal lung tissue has very low levels of expression [20]. In comparison to type II alveolar cells that produce surfactants and alveolar macrophages, bronchial epithelial cells exhibited a greater quantity of TMPRSS2. Type 1 alveolar cells, which line the respiratory tract, do not express this gene [21, 22].

Genetic research has demonstrated that the 5' untranslated region (UTR) of the TMPRSS2 gene harbors an androgen response element (ARE). Androgenic hormones can control transcription, among their many other functions [23,24]. The human TMPRSS2 gene, which has fourteen exons and thirteen introns, is located on chromosome 21 [25].

TMPRSS2 structure

Animals that carry the TMPRSS2 gene include zebrafish, chimpanzees, dogs, cows, monkeys, rats and mice. There are some variations, but it's still very similar to the human enteropeptidase gene. The protein contains a type II transmembrane domain and an LDL receptor class A (LDLRA) domain that can bind calcium, an SRCR domain that interacts with extracellular and intracellular molecules, The protein has a serine protease domain belonging to the S1 family, which specifically cleaves at arginine or lysine residues [26].

TMPRSS2 Physiology:

There are a number of substrates that could TMPRSS2 work with. Donaldson and his colleagues investigated the control of sodium ion efflux through the epithelial sodium channel (ENaC). They found that TMPRSS2 expression decreased this outflow [27]. This suggested that protease modulated the ENaC, albeit it was not apparent if this was exclusive to TMPRSS2.

Besides, experiments conducted under controlled conditions showed that TMPRSS2 can specifically target the protease-activated receptor-2 (PAR-2) in a prostate cancer cell

line that relies on androgen for growth [28]. The activation of PAR-2 protease caused intracellular calcium ion reserves to be released, which in turn started downstream signaling [29]. Excessive TMPRSS2 expression in prostate cancer cells activates the TSP matrilysin, which in turn stimulates PAR-2, according to the research [30].

Role of TMPRSS2 in Viral Entry

Enveloped viruses, including the flu and coronavirus, bind their glycoproteins (GP) to specific receptors in order to enter host cells. In order to bind to the cell membrane, several viral glycoproteins employ proteolytic activity. The exact moment and place where proteolytic activation occurs varies among viruses. Certain glycoproteins are cleaved upon release from the host cell, whereas other glycoproteins cleave in two different stages, either prior to or subsequent to the virus merging with a new host cell [31].

The proteases that are utilized are determined by the cleavage motifs found on GPs [32]. If these patterns are altered, the virus's infectiousness and tropism could be significantly affected. This has been observed in numerous respiratory viruses. The presence of TMPRSS2 is necessary for the activation of respiratory viruses, such as influenza and coronaviruses. In human airways, TMPRSS2 is highly expressed [33].

Angiotensin-converting enzyme 2 (ACE2)

ACE2 is an essential enzyme within the renin-angiotensin-aldosterone system (RAAS), which is pivotal in maintaining blood pressure and fluid equilibrium within the body [34]. In addition, ACE2 functions as a receptor for both the SARS-CoV and SARS-CoV-2 viruses, aiding their penetration into host cells, particularly within the respiratory system. More precisely, the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2 contains a segment that attaches to ACE2 [35].

The membrane-bound protein ACE2 is expressed in several human organs, such as the kidneys, heart, intestines, and lungs. The discovery that this protein is found on the surface of endothelium, lung alveolar epithelial, and ciliated bronchial cells is noteworthy. Furthermore, ACE2 expression in the mucosa of the oral cavity was confirmed using *in silico* analysis of RNA-seq profiles [36].

Function:

During the protective phase of the RAAS, the activity of ACE is inhibited by soluble angiotensin-converting enzyme 2 (sACE2). ACE, which is produced during the detrimental stage of the RAAS, converts angiotensin I to angiotensin II, prompting a series of hormonal reactions that lead to hypertension. ACE2's antagonistic effect leads to a decrease in blood pressure by converting angiotensin II into angiotensin by eliminating the carboxyl-terminal phenylalanine [37, 38].

Expression pattern of ACE and ACE2 in human disorders

Ace2 serves as the receptor by which SARS-CoV gains entry into cells, while also providing protection to the lungs. The results show that ACE2 expression is a significant marker of SARS-CoV infection. When this protein is involved, the severity of co-occurring disorders can be affected [39,40]. The expression pattern of ACE and ACE2's involvement in human illnesses is summarized in Table (1).

Table 1. Expression pattern of ACE and ACE2 in human disorders (41).

Disease	Expression and engagement		Clinical samples	Function
	ACE	ACE2		
infection with SARS-CoV	-	↑	Membranes lining the airways and lungs of humans	Acute respiratory syndrome coronavirus mostly infects ACE2-expressing specialized ciliated epithelial cells. SARS-CoV with an enhanced S protein was able to infect 293 T cells with transitory ACE2 overexpression. Keeping p38MAPK, ERK, and JNK expression levels consistent by hyperphosphorylation
	-	↑	The 293 T kidney cells of a human (hu)	
	↑	↓	Rat with diabetes caused by STZ NRK-52E cells with elevated glucose levels	
	↓	↓	Kidney of a Sprague-Dawley rat with diabetes	
Diabetes	↑	↓	Tissue samples from 20 people diagnosed with type 2 diabetes were obtained from their kidneys.	Patients with type 2 diabetes who also suffer from nephropathy are more likely to have an elevated ACE/ACE2 ratio, which is associated with kidney damage. In hypertensive brains, the ACE2/Ang MasR pathway is defective, while the ACE/Ang II/AT1 route is active. Angiotensin II inhibits ACE/ACE2 activity through the AT1-ERK/p38 pathway on both the molecular and protein levels.
	↑	↓	SHR rats	
	↑	↓	Hypertensive human kidney/heart refers to the medical condition characterized by elevated blood pressure that specifically impacts the kidneys and heart in humans.	
	-	↓	The kidneys of hypertensive rats, specifically the spontaneously hypertensive rat (SHR) and the Wistar-Kyoto (WKY) rat, were studied.	
Hypertension	-	↑	Male Sprague-Dawley rats	While AT2R and Mas expression rose, AT1R and ACE expression fell while ACE2 expression rose. As a result, there was a significant decrease in the levels of the proinflammatory cytokines TNF- α , IL-1 β , and IL-6 in the PVN. Coronal ACE activity elevated, medulla and cortex ACE2 activity decreased, plasma and urine ACE2 activity increased
	↑	↓	STNx refers to subtotal nephrectomy, a surgical procedure involving the removal of a portion of the kidney in rats.	
Kidney disease	↑	↑	There were 78 samples of renal cortex.	The concentration of Ang II in a certain region is inversely correlated with the expression of the ACE and ACE2 genes. activity is associated with an elevated risk of cardiovascular mortality and major adverse cardiovascular events (MACE).
	-	↑	There is a total of 79 individuals diagnosed with obstructive coronary artery disease (CAD).	
Cardiovascular	-	↑	The cardiac and renal tissues of GHR ^{-/-} mice	The situation deteriorates for the ACE2/Ang-2/Mas receptor axis.
	-	↑		

Table 1 continue

Disease	Expression and engagement		Clinical samples	Function
	ACE	ACE2		
	↑	↑	Rat with myocardial infarction	In myocardial infarction, increased levels of ACE and ACE2 were seen in both the border/infarct zone and the viable myocardium.
	-	↑	Human heart failure, sometimes referred to as idiopathic dilated cardiomyopathy (IDC) and ischemic cardiomyopathy (ICM).	In human IDC and ICM, ACE2 expression is elevated.
		↓	The study focused on analyzing the BALF (bronchoalveolar lavage fluid) and lung tissue of rats with LPS-induced ARDS (acute respiratory distress syndrome).	One way AEC2 reduces LPS-induced ARDS is by blocking the Ang-1/Mas pathway, which activates ERK/NF-κB.
Acute respiratory distress syndrome (ARDS)	-	-	There were 31 patients with Acute Respiratory Distress Syndrome (ARDS), of which 51% survived.	Survivors exhibit elevated levels of ACE/ACE2 activity.
Acute lung injury (ALI)		↓	Rats experiencing acute lung injury (ALI) induced by lipopolysaccharide (LPS)	The LPS group demonstrated a notable decrease in ACE2 and VDR mRNA levels compared to the control group without any treatment, and this reduction was statistically significant.
	↑	↓	Rats were induced with acute lung damage using lipopolysaccharide (LPS) to stimulate pulmonary microvascular endothelial cells (PMVECs).	Up-regulated ACE/Ang II/AT1R axis
Neonatal pulmonary damage	-	↓	ACE2 knockout ALI-induced mice	While rhuACE2 protected animals against acute lung injury, ACE2 depletion resulted in severe signs of acute lung injury.
	-	↓	A549 cells are alveolar epithelial cells.	The existence of meconium proteolytic enzymes led to a decrease in the defensive function of ACE-2 expression in human A549 cells.
Smoking	-	↑	Human lung tissue	Individuals that smoke may have a higher vulnerability to novel coronavirus that emerged in 2019.
Inflammatory bowel disease (IBD)	-	↑	CD, UC patients with IBD	Angiotensin, ACE2 enzyme activity, and the ratio of ACE to ACE2 were all elevated in patients with inflammatory bowel illness.

STZ (a glucosamine-nitrosourea compound derived from Streptomyces achromogenes), **P38MAPK** (mitogen-activated protein kinases), **ERK** (Extracellular signal-regulated kinase) **JNK** (The c-Jun N-terminal kinase), **NRK** (a rat kidney cell line exhibiting epithelial morphology that was isolated from a normal adult rat), **SHR** (Spontaneously hypertensive rat), **AT1R** (Angiotensin type 1 receptor), **AT2R** (Angiotensin type 2 receptor), **CAD** (Coronary Artery Disease), **GHR** (Growth Hormone Receptor), **LPS** (Lipopolysaccharides), **VDR** (vitamin D receptor), **IDC** (Invasive ductal carcinoma), **ICM** (Ischemic cardiomyopathy), **BALF** (bronchoalveolar lavage fluid).

Structure:

There are multiple metalloenzymes present in renal tubular cells and intestinal enterocytes. One such example of this is the membrane-bound angiotensin-converting enzyme 2 (mACE2). The terminal domains of the mACE2 protein contain the peptidase M2 domain and the amino acid transporter collectrin [42].

Intestinal cells, similar to cells in other organs, possess the enzymatically active region of mACE2. The protein is a type I membrane protein that spans the membrane once. During the body's defensive stage, the enzyme ADAM17 has the ability to cleave the extracellular domain of mACE2 from its transmembrane domain. This method facilitates the management of blood pressure. The expression "soluble ACE2" describes the amount of ACE2 that has hydrolyzed. By binding to MasR receptors, this leads to localized vasodilation and, as a result, reduces blood pressure. Eventually, it is possible that urine might remove excess sACE2 [43–44].

ACE2 has potential for use in the treatment of COVID-19:

To infect host cells, SARS-CoV-2 relies on an essential receptor called ACE2. Research has demonstrated that ACE2 serves as the primary functional receptor for SARS-CoV2 to gain entry into cells [2]. The procedure is as follows S1 and S2 subunits are generated when the surface S protein of the virus cleaves at various points during its growth. Cell membrane fusion and receptor recognition both require these subunits. S1 subunit-ACE 2 communication occurs through receptor binding domain. The host protease binds to S2, exposing yet another cleavage site, and this causes the protein to cleave [45].

In the absence of this process, the virus is unable to initiate its invasion. Viruses impact both the ACE-AngII/AT1R and ACE2-Ang1-7/Mas axis by decreasing the amounts of ACE2 protein. . The increased activation of AT1R receptors brought on by the abrupt rise in AngII levels is what starts the acute lung failure phase. This condition is characterized by an elevated permeability of the capillaries. This is where we present a scientific explanation for the mechanism by which a coronavirus-induced acute respiratory distress syndrome (ARDS) leads to death [46].

An effective strategy to battle SARS-CoV-2 involves inhibiting the interaction between the virus and human ACE2. Targeting the functional receptor ACE2 or the receptor binding domain (RBD) of the viral S protein will accomplish this. Additional treatment options include the application of small molecule inhibitors, antibodies against ACE2, peptides generated from ACE2, or single-chain antibody fragments that specifically target ACE2 [45]. However, blocking ACE2 might have harmful effects on different organs.

Single-nucleotide polymorphisms (SNPs) in ACE2

Scientists have investigated potential links between diseases and certain genetic variations called single-nucleotide polymorphisms (SNPs) in various ethnicities. There is

growing evidence that single nucleotide polymorphisms (SNPs) impact gene expression, which in turn influences health consequences. Because association research findings might differ greatly based on demographic variables including age, race, and selection criteria, disputes within groups may arise. Research on the relationship between ACE2 SNPs and conditions such cerebral malaria, essential hypertension, dyslipidemia, hypertrophic cardiomyopathy, and ventricular hypertrophy has been done in a variety of populations [47].

Interestingly, research demonstrated that reducing promoter methylation increased ACE2 expression by allowing immune cells to enter certain tumor cells. It would be helpful to find any functional SNPs that may affect the susceptibility of the population [48].

CONCLUSION

This study provides valuable insights into the emergence and characteristics of the SARS-CoV-2 outbreak, from its initial identification to the mechanisms involved in cellular entry. By tracing the origins of the virus and elucidating its entry mechanisms, we contribute to a deeper understanding of this global health crisis. In addition, epithelial cell ACE2 and TMPRSS2 expression is linked to viral entry and their association with human co-morbidities should be analyzed. While our study sheds light on the emergence and characteristics of the SARS-CoV-2 outbreak and its cellular entry mechanisms, several limitations warrant acknowledgment. The study focused primarily on the early stages of the SARS-CoV-2 outbreak, potentially overlooking detailed variations and mutations that took place in viral structure over time. In addition, development of anti-viral medications and vaccines were not addressed.

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