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Replication of corona virus &its mechanism in host cell entry mediated

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Abstract- Corona virus (Cov) are the causative agent of respiratory & enteric disease in animal ,human & bird . Three human corona virus are known to exist Human corona 229E (HCoV -229E) HCoV-OC43 & SARS SARS which caused a worldwide health threat in 2003. SARS associated with fever cough & respiratory complication illness causes more than 15% mortality world wide .So far there is no remedy for the illness except supportive treatment In corona virus the structural protein N (Nucleocapsid protein) associated with the viral RNA to form the filamentous nucleocapsid& play a crucial role in genome replication & transcription Corona enveloped positive sense RNA virus which are characterized by spike with large RNA genome & its unique replication strategy In this review discuss a introduction of corona virus & their replication, pathogenesis , prevention& treatment

Keywords-Nucleocapsid, protein, positive sense RNA virus, HCoV-OC43,Corona virus,SARS

Introduction

Coronaviruses are the largest group of viruses belonging to the *Nidovirales* order, *Coronaviridae,Arteriviridae*, and *Roniviridae* families. The *Coronavirinae* belong to one of two subfamilies in the *Coronaviridae* family, with

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the other being the *Torovirinae*. The *Coronavirinae* are further subdivided into four groups, the alpha, beta, gamma and delta coronaviruses. Viruses belongs to *Nidovirales*order are enveloped, non-segmented positive-sense RNA viruses. They contain very large genomes for all the RNA viruses *Coronavirinae* having the largest identified RNA genomes, containing 30 kilobase (kb) genomes. Other features are(i) conserved genomic organization, with large replicase gene , structural and accessory genes(ii) Many nonstructural genesexpressionl(iii) Unique or unusual enzymatic activities encoded within the large replicase-transcriptase polyprotein

Corona virus genomic organization-

Coronaviruses contain a non-segmented, positive-sense RNA genome of 30 kb. The genome contains a 5′ cap structure with a 3′ poly (A) tail, a act as a mRNA for translation of the replicasepolyproteins. The replicase gene encoding the nonstructural proteins (Nsps) which is two-thirds of the genome, about 20 kb, as opposed to the structural and accessory proteins, which make up only about 10 kb of the viral genome. The 5′ end of the genome contains a leader sequence and untranslated region (UTR) that contains multiple stem loop structures required for RNA replication and transcription., At the beginning of each structural or accessory gene are transcriptional regulatory sequences (TRSs) that are required for expression of each of these genes The 3′UTR also contains RNA structures required for replication and synthesis of viral RNA. The organization of the coronavirus genome is 5′-leader-UTR-replicase-S (Spike)–E

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(Envelope)-M (Membrane)-N (Nucleocapsid)-3′UTR-poly (A) tail with accessory genes interspersed within the structural genes at the 3′ end of the genome. The accessory proteins are non-essential for replication in tisSequencing analysis of the virion genomic RNAs reveals the presence of 10 open reading frames. These ORFs encode various structural and nonstructural proteins of coronaviruses.(1)

Virion Structure

Coronavirus virions are spherical, enveloped virus particles, ranging from 80 to 160 nm in diameter. Prominent surface projections of up to 20 nm in length cover the entire virion surface (117), giving it the corona appearanceInside the envelope resides a helical nucleocapsid of 6-8 nm in diameter),Helical nucleocapsid is associated with viruses containing a negative-stranded RNA genome, coronavirus contains a positive-stranded RNA genome Typically, positive-stranded RNA viruses have icosahedral nucleocapsids.. All coronavirus virion particles contain three to four structural proteins.1 spike protein (S; old terms: E2 or gpI80), which constitutes the spikes, or peplomers, on the virion envelope. with 180 kd weight and is frequently cleaved into two proteins of 90 kd(2).2Glycoprotein is that it shares some sequence homology with the hemagglutinin protein of influenza C virus .3 , HE protein of also exhibits hemagglutinating and esterase actIvity HE protein may play a significant role in coronavirus biology.

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4 Structural protein is an internal component of the virus. This protein, N, is a phosphoprotein of 50 kd), which constitutes the nucleocapsidprote:in of the virus. The protein binds to virion RNA, providing the structural basis for the helical nucleocapsid

Entry of corona virus in host Attachment and Entry

The initial attachment of the virion to the host cell is initiated by interactions between the S protein and its receptor. The sites of receptor binding domains (RBD) within the S1 region of a coronavirus S protein vary depending on the virus, with some having the RBD at the N-terminus of S1 (MHV), while others (SARS-CoV) have the RBD at the C-terminus of S1 (3 4), Many coronaviruses utilize peptidases as their cellular receptor. peptidases are used, as entry occurs even in the absence of the enzymatic domain of these proteins. Many α-coronaviruses utilize aminopeptidase N (APN) as their receptor, SARS-CoV and HCoV-NL63 use angiotensin-converting enzyme 2 (ACE2) as their receptor,).

Following receptor binding, the virus gain access to the host cell cytosol. This is generally accomplished by acid-dependent proteolytic cleavage of S protein by a cathepsin, TMPRRS2 or another protease, followed by fusion of the viral and cellular membranes. S protein cleavage occurs at two sites within the S2 portion of the protein, with the first cleavage important for separating the RBD and fusion domains of the S protein [5] and the second for exposing the fusion peptide (cleavage at S2′). Fusion occurs within acidified endosomes, Cleavage at S2′

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exposes a fusion peptide that inserts into the membrane, which is followed by joining of two heptad repeats in S2 forming an antiparallel six-helix bundle The formation of this bundle allows for the mixing of viral and cellular membranes, resulting in fusion and ultimately release of the viral genome into the cytoplasm.

Replication

Coronavirus RNA synthesis occurs independently of DNA-dependent RNA synthesis. Thus, the coronavirus-specific RNA synthesis in the infected cells is usually studied in the presence of actinomycin D, which inhibits host RNA synthesis. Depending on the cell lines and viruses involved, virus-specific RNAs can usually be detected a few hours after virus infections

Viral RNA synthesis follows the translation and assembly of the viral replicase complexes. Viral RNA synthesis produces both genomic and sub-genomic RNAs. Sub-genomic RNAs serve as mRNAs for the structural and accessory genes which reside downstream of the replicasepolyproteins. All positive-sense sub-genomic RNAs are 3′ co-terminal with the full-length viral genome . Both genomic and sub-genomic RNAs are produced through negative-strand intermediates. These negative-strand intermediates are only about 1 % as abundant as their positive-sense counterparts and contain both poly-uridylate and anti-leader sequences

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Many cis-acting sequences are important for the replication of viral RNAs. Within the 5′ UTR of the genome are seven stem-loop structures that may extend into the replicase 1a gene (6,) The 3′ UTR contains a bulged stem-loop, a pseudoknot, and a hypervariable region Interestingly, the stem-loop and the pseudoknot at the 3′ end overlap, and thus cannot form simultaneously Therefore, these different structures are proposed to regulate alternate stages of RNA synthesis, although exactly which stages are regulated and their precise mechanism of action are still unknown.

Finally, coronaviruses are also known for their ability to recombine using both homologous and nonhomologous recombination]. The ability of these viruses to recombine is tied to the strand switching ability of the RdRp.(7) Recombination likely plays a prominent role in viral evolution and is the basis for targeted RNA recombination, a reverse genetics tool used to engineer viral recombinants at the 3′ end of the genome.

Enzyme involved in RNA

- Coronaviruses do not carry RNA polymerases in the virion. Therefore, the RNA polymerases used for RNA transcription have to be synthesized de novo from the incoming virion genomic RNA initially and then from the newly synthesized mRNAs later in the infection. Indeed, the inhibitors of protein synthesis such as cycloheximide,

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when added to the infected cells immediately after infection, inhibit RNA synthesis Thedependency of RNA synthesis on protein synthesis continues throughout the viral replication cycle. Thus, either RNA polymerases or some cofactors have to be continuously synthesized. Two types of RNA-dependent RNA polymerase activities specific for coronaviruses have been detected in the lysates of several coronavirusinfected cells enzymatic properties, including pH optimum and cationic requirements Corona virus 323 active protein synthesis The first or early polymerase is most likely responsibk for negative-stranded RNA synthesis, while the second or late polymerase is responsible for the positive-stranded RNA synthesis

Pathogenesis

1 **Animal Coronaviruses**

Coronaviruses cause a large variety of diseases in animals, such as pigs, cows, chickens, dogs, and cats Transmissible Gastroenteritis Virus (TGEV) and Porcine Epidemic Diarrhea Virus (PEDV) cause severe gastroenteritis in young piglets, Porcine hemagglutinating encephalomyelitis virus (PHEV) mostly leads to enteric infection but has the ability to infect the nervous system, causing encephalitis, vomiting, and wasting in pigs. Feline enteric coronavirus (FCoV) causes a mild or asymptomatic infection in domestic cats, Bovine CoV, Rat CoV, and

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Infectious Bronchitis Virus (IBV) cause mild to severe respiratory tract infections in cattle, rats, and chickens, respectively..

2 **Human Coronaviruses**

HCoV-229E &.HCoV-OC43 were identified in the mid 1960 are known to cause the common cold(7 ,8,9,) The recently identified HCoV19 cause a pneumonia & is the most pathogenic virus Human corona virus have been associated with common cold diarrhea

Diagonis& Treatment Prevention

, Diagnosis of coronaviruses is unnecessary, as the disease will naturally run its course.. Diagnosis is also important in locations where a severe CoV outbreak is occurring, such as, at present, in the Middle East, where MERS-CoV continues to circulate. The identification of cases will guide the development of public health measures to control outbreaks. It is also important to diagnose cases of severe veterinary CoV-induced disease, such as PEDV and IBV, to control these pathogens and protect food supplies. RT-PCR has become the method of choice for diagnosis of human CoV,.

Only limited options are available to prevent coronavirus infections. Vaccines have only been approved for IBV, TGEV, and Canine CoV, but these vaccines are not always used because they are either not very effective, or in some cases have been reported to be involved in the

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selection of novel pathogenic CoVs via recombination of circulating strains. Vaccines for veterinary pathogens, such as PEDV, may be useful in such cases where spread of the virus to a new location could lead to severe losses of veterinary animals. In the case of SARS-CoV, several potential vaccines have been developed but none are yet approved for use. These vaccines include recombinant attenuated viruses, live virus vectors, or individual viral proteins expressed from DNA plasmids. Therapeutic SARS-CoV neutralizing antibodies have been generated and could be retrieved and used again in the event of another SARS-CoV outbreak. Such antibodies would be most useful for protecting healthcare workers. In general, it is thought that live attenuated vaccines would be the most efficacious in targeting coronaviruses. This was illustrated in the case of TGEV, where an attenuated variant, success, Vaccine development for coronaviruses faces many challenges that significantly attenuate the virus Owing to the lack of effective therapeutics or vaccines, the best measures to control human coronaviruses remain a strong public health surveillance system coupled with rapid diagnostic testing and quarantine when necessary. For international outbreaks, cooperation of governmental entities, public health authorities, and health care providers is critical. During veterinary outbreaks that are readily transmitted, such as PEDV, more drastic measures such as destruction of entire herds of pigs may be necessary to prevent transmission of these deadly viruses.

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Conclusion

Coronavirus RNA synthesis and gene expression involve very unique mechanisms. The leader-primed transcription and high-frequency RNA recombination are features not observed in any other systems.

Over the past 50 years the emergence of many different coronaviruses that cause a wide variety of human and veterinary diseases has occurred. It is likely that these viruses will continue to emerge and to evolve and cause both human and veterinary outbreaks owing to their ability to recombine, mutate, and infect multiple species and cell types.

Future research on coronaviruses will continue to investigate many aspects of viral replication and pathogenesis. First, understanding the propensity of these viruses to jump between species, to establish infection in a new host, and to identify significant reservoirs of coronaviruses will dramatically aid in our ability to predict when and where potential epidemics may occur. As bats seem to be a significant reservoir for these viruses, it will be interesting to determine how they seem to avoid clinically evident disease and become persistently infected. Second, many of the non-structural and accessory proteins encoded by these viruses remain uncharacterized with no known function, and it will be important to identify mechanisms of action for these proteins as well as defining their role in viral replication and pathogenesis. These studies

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should lead to a large increase in the number of suitable therapeutic targets to combat infections. Furthermore, many of the unique enzymes encoded by coronaviruses, such as ADP-ribose-1″-phosphatase, are also present in higher eukaryotes, making their study relevant to understanding general aspects of molecular biology and biochemistry. Third, gaining a complete picture of the intricacies of the RTC will provide a framework for understanding the unique RNA replication process used by these viruses. Finally, defining the mechanism of how coronaviruses cause disease and understanding the host immunopathological response will significantly improve our ability to design vaccines and reduce disease burden.

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