MERS-CoV: Identifying Viral and Host Factors Associated with Transmission, Pathogenesis, and Treatment Development

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SUMMARY

Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel betacoronavirus that is associated with a high mortality rate (~36%) and is endemic in the Middle East. Since 2012, there have been 2100 laboratory confirmed cases and 730 deaths associated with MERS-CoV in 27 countries. Clinical manifestations of the virus range from asymptomatic to acute respiratory distress syndrome (ARDS), septic shock, and renal failure resulting in death. Bats are the natural reservoirs of MERS-CoV and can travel over long distances to transmit the virus to different intermediate hosts and humans. This, combined with the MERS-CoV spike (S) protein’s ability to undergo mutations that increase its transmission efficiency, gives the virus a pandemic potential. Currently there is a lack of knowledge on how viral and host factors contribute to the transmission and pathogenesis of MERS-CoV in humans. This article explores the different viral and host factors associated with MERS-CoV transmission and pathogenesis, and how this knowledge can aid in the development of therapeutics. MERS-CoV transmission is facilitated by interaction between the viral receptor binding domain of the S protein and the host receptor dipeptidyl peptidase 4 (DPP4). MERS-CoV can utilize multiple host proteases such as furin, transmembrane serine protease 2 (TMPRSS2), trypsin, and cathepsin to cleave the S protein, which facilitates membrane fusion. MERS-CoV has been shown to primarily infect cells in the lower respiratory tract of humans, but it can also infect immune and kidney cells. An overactive inflammatory response, induction of apoptosis, and cytokine dysregulation are vital factors that contribute to the high pathogenicity associated with MERS-CoV infection. Future treatment options for MERS-CoV include inhibition of virus entry, fusion, polyprotein cleavage, and replication by targeting both viral and host factors.

INTRODUCTION

Middle East respiratory syndrome coronavirus (MERS-CoV) is a highly pathogenic human coronavirus, which was first identified in the Middle East in 2012 [1, 3]. It belongs to the Coronaviridae family and the C lineage within the Betacoronavirus genus.
Like other coronaviruses, MERS-CoV is an enveloped virus with a positive-sense single-stranded RNA genome. Its genome consists of over 30,000 nucleotides and encodes for non-structural proteins, accessory proteins, and structural proteins including the spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins [1, 4]. Since 2012, there have been 2100 laboratory confirmed cases and 730 deaths associated with MERS-CoV in 27 countries, with an alarming fatality rate of ~36% [2]. Clinical manifestations of the virus range from asymptomatic to acute respiratory distress syndrome (ARDS), septic shock, and renal failure resulting in death [5, 6]. The severity of disease and fatality rates are much higher in patients with existing co-morbidities such as diabetes and hypertension [5]. The natural reservoir for MERS-CoV is thought to be bats in the Vespertilionidae family [1]. This observation is supported by studies demonstrating that bat coronaviruses phylogenetically related to MERS-CoV (HKU4 and HKU25) were able to utilize the MERS-CoV receptor, human dipeptidyl peptidase 4 (hDPP4), for cell entry [7, 8]. The virus is proposed to have been transmitted from bats to dromedary camels, which acted as an intermediate host and allowed for the diversification of MERS-CoV through multiple recombination events and mutations in the S protein, which is involved in viral attachment and entry [9].

Unlike humans, the presence of the virus in bats and camels does not lead to overt disease signs [12]. Studies have shown that alpacas, non-human primates, goats, cows, sheep, and horses could act as potential intermediate hosts for MERS-CoV as well [1, 3]. MERS-CoV is primarily transmitted from camels to humans through respiratory secretions, saliva, urine, and raw camel meat and milk consumption [9, 10]. In addition, limited human-to-human transmission through respiratory secretions, direct close contact, and fomites has been observed mostly in hospital settings [11]. Further mutations in the receptor binding domain of the S protein could increase the transmissibility of MERS-CoV among humans [1]. The ability of infected bats to fly long distances and the high mutations rates associated with the MERS-CoV S protein make it likely to cause the next global outbreak [4]. Thus, it is essential to understand the viral and host factors that contribute to the transmission and pathogenesis of MERS-CoV in humans. A better understanding of these viral and host factors will allow for the development of potential direct-acting antivirals (DAAs) and indirect-acting antivirals (IAAs) to treat patients.

RESEARCH QUESTIONS

Bats are the natural reservoirs of MERS-CoV and can travel over long distances to transmit the virus to different intermediate hosts and humans. Because of urbanization and deforestation, bats are encroaching into human territory more frequently [13]. This, combined with the ability of MERS-CoV S protein to undergo mutations that increase its transmission efficiency, give the virus a pandemic potential. Currently, there is a lack of knowledge on how viral and host factors contribute to the transmission and pathogenesis of MERS-CoV in humans. MERS-CoV infection is also associated with an extremely high fatality rate of ~36%, and presently there are no treatments available to treat infected patients [1, 2]. Determining the exact molecular mechanisms by which viral and host factors contribute to MERS-CoV transmission and pathogenesis may help prevent a future global outbreak. Furthermore, it will open new avenues on how to develop DAAs and IAAs to treat MERS-CoV. Three questions need to be addressed to better understand how MERS-CoV infection leads to disease and mortality in humans. First, the viral and host factors associated with MERS-CoV transmission need to be elucidated. Then, it is crucial to understand the role that viral and host factors play in the pathogenesis related to MERS-CoV. Finally, it is important to investigate the potential DAAs and IAAs that can be used to target viral and host factors in MERS-CoV infected patients. By answering these three questions, this article aims to address the gap in knowledge that currently exists in our understanding of MERS-CoV transmission, pathogenesis, and potential treatment options.

PROJECT NARRATIVE

Viral and host factors associated with MERS-CoV transmission

A fundamental requirement for the successful transmission of viruses is their ability to utilize both viral and host proteins to facilitate attachment, entry, and replication in the host
For MERS-CoV, effective transmission involves the critical interaction between the viral S protein and host cell DPP4 receptor [15, 16]. The S protein consists of the S1 subunit, which contains the receptor binding domain (RBD) involved in binding DPP4, and the S2 subunit, which carries the fusion peptide involved in membrane fusion [15, 16]. Although DPP4 is relatively conserved between mammalian species, differential glycosylation patterns and differences in amino acid residues involved in binding the S protein act as barriers to MERS-CoV transmission [3, 17, 18]. The ability of MERS-CoV to be transmitted through droplets makes it highly contagious, but, surprisingly, there is limited human-to-human transmission. This is because DPP4 is predominantly expressed in the lower respiratory tract, but not in the upper respiratory tract of humans [10]. DPP4 is located in the upper respiratory tract of dromedary camels, thus allowing the efficient transmission of MERS-CoV to camels via droplet secretions [10]. Like the avian influenza A/H5N1 virus, MERS-CoV may be a few mutations away from being able to utilize receptors in the upper respiratory tract of humans, which would result in increased transmission efficiency and also increase the risk of a future global pandemic [19]. During the 2015 South Korea MERS-CoV outbreak, a single point mutation in the S1 RBD reduced the affinity of the virus to human DPP4 but increased its transmissibility [20]. The increased transmissibility could be explained by polymorphisms in DPP4, increased MERS-CoV replication in the submucosal glands of the upper respiratory tract, and better evasion of the host immune system [20].

The proteolytic cleavage of the viral envelope glycoproteins permits membrane fusion, and it is an essential step for efficient viral transmission [21]. Unlike other human coronaviruses, the S protein in MERS-CoV is cleaved during its biosynthesis [21]. A two-step activation mechanism has been proposed for MERS-CoV (Fig. 1) [21, 22]. The S protein is first cleaved between S1 and S2 subunits by furin in the endoplasmic reticulum-Golgi compartments of the producer cell. This early cleavage is required for infection of human lung cells but is not necessary to infect other cell types [21]. This cleavage allows the RBD of S1 to attach to the target cell. Once attached to the target cell, a second cleavage occurs upstream of the S2 subunit at the host cell surface [21, 22]. This cleavage by host cell transmembrane serine protease 2 (TMPRSS2), furin or trypsin exposes the fusion peptide, which facilitates membrane fusion and the release of the viral genome into the cytoplasm. Without the first cleavage event, the uncleaved MERS-CoV is endocytosed into the target cell [14].
cell and cleaved by Cathepsin L and/or B in endosomes [21]. Uncleaved MERS-CoV is less infectious to human airway epithelial cells and can only infect cells containing late-acting endosomal proteases.

**Viral and host factors associated with MERS-CoV pathogenesis**

The ubiquitous expression of DPP4 in different cell types and the ability of MERS-CoV to use multiple host cell proteases to cleave the S protein helps explain the broad cell tropism and increased pathogenicity associated with the virus (Fig. 2) [22, 23]. The natural ligand for DPP4 is adenosine deaminase (ADA), and their interaction plays a significant role in the proliferation, and activation of T cells [24]. MERS-CoV hijacks the host DPP4 receptor, thus preventing ADA binding and limiting T cell activation. The lower respiratory tract is the primary site for MERS-CoV infection in humans [10]. MERS-CoV has been shown to robustly infect and replicate in non-ciliated bronchial epithelial cells, bronchiolar epithelial cells, alveolar epithelial cells, and endothelial cells of pulmonary vessels [25]. Upon infection, these cells undergo apoptosis through extensive caspase-3 activation [25]. This increased apoptosis possibly contributes to pneumonia and acute lung injury observed in MERS-CoV infected patients. Papain-like protease (PLpro), M protein, and accessory proteins 4a, 4b, and 5 are used by MERS-CoV to evade the host immune system through interferon suppression, deubiquitinating and deISGylating activities [26].

Infection of alveolar macrophages and dendritic cells by MERS-CoV leads to the release of proinflammatory cytokines and chemokines [26]. The persistent expression of proinflammatory cytokines recruit and activate neutrophils, which damage infected tissues [27]. One study showed that although immunosuppressed macaques support higher levels of MERS-CoV replication in respiratory tissues, the pathology in their lungs was significantly lower compared to non-immunosuppressed macaques [28]. This demonstrates that MERS-CoV itself causes little damage to the infected cells and pathogenesis can be attributed to an overactive inflammatory response.

**FIG. 2 Viral and host factors contributing to MERS-CoV pathogenesis:** This figure is a summary of the major viral and host factors associated with MERS-CoV pathogenesis in humans.
T cells play a significant role in controlling MERS-CoV, but the virus is capable of infecting and inducing T cell apoptosis through the intrinsic and extrinsic apoptotic pathways [27]. Furthermore, infection of T cells results in the dysregulation of cytokine production, which can lead to a cytokine storm and septic shock in patients [27]. MERS-CoV downregulates antigen-presentation pathways, which results in decreased T-cell activation to fight MERS-CoV infection [27]. The ability of MERS-CoV to infect pulmonary endothelial cells, dendritic cells, and T cells allows the virus to disseminate beyond the respiratory tract and infect other organs, such as the kidneys [25, 27]. MERS-CoV has been shown to induce apoptosis in lung and kidney cells through the upregulation of proapoptotic proteins, Smad7 and fibroblast growth factor (FGF2) [29]. The extensive apoptosis in the lung and kidney cells contribute to the ARDS, and renal failure observed in infected patients.

**Potential DAAs and IAAs for the treatment of MERS-CoV**

Given the current knowledge we possess about viral and host factors contributing to MERS-CoV transmission and pathogenesis, different DAAs and IAAs could potentially be developed for treatment purposes (Fig. 3).

DPP4 inhibitors which target the binding interface between DPP4 and the RBD of S1 can be effective at preventing MERS-CoV attachment and entry [30]. ADA, the natural ligand for DPP4, has been shown to act as a natural antagonist for MERS-CoV binding [31]. Human monoclonal antibodies, such as m336, have been shown to effectively neutralize the MERS-CoV S protein [32]. Furthermore, soluble DPP4 can potentially be used to prevent the S1 RBD from binding to cell-surface DPP4.

The viral fusion process can be blocked by either targeting the host cell proteases or the S2 domain of MERS-CoV. A peptide, called HR2P, has been successful in inhibiting the fusion process during MERS-CoV infection [33]. Inhibitors of host cell proteases such as furin, TMPRSS2, and cathepsins can be used not only to treat MERS-CoV infection, but also other enveloped viruses. Potent synthetic inhibitors of furin have been developed, which could potentially be used for the treatment of infectious enveloped viruses, including MERS-CoV [34]. Furthermore, the use of furin-directed human microRNA, miR-24, can be utilized to downregulate furin expression [35]. A robust decrease in both furin activity and mRNA levels were observed using miR24 mimics. This decrease in furin activity led to a significant reduction in influenza H5N1 virions and completely blocked viral spread. Given the significance of furin-mediated cleavage of the MERS-CoV S protein, miR24 mimics may play a pivotal role in preventing the fusion process. A potent TMPRSS2 inhibitor also

**FIG. 3 Potential DAA and IAA targets for the treatment of MERS-CoV:** This figure is a summary of the potential DAA and IAA targets for the treatment of MERS-CoV. The four categories for therapeutics include entry, fusion, protease, and replicase inhibitors directed towards both the virus and the host [30].
demonstrated efficient blockage of the influenza fusion process in human airway epithelial cells [36]. Teicoplanin, a glycopeptide antibiotic, has been shown to potently block the entry of MERS-CoV, severe acute respiratory syndrome coronavirus (SARS-CoV) and, Ebola by specifically inhibiting cathepsin L activity [37].

3C-like protease (3CLpro) and PLpro are viral proteases which play a critical role in MERS-CoV replication by cleaving the viral polypeptide following translation. A covalent low molecular inhibitor was shown to inhibit the activity of PLpro for both MERS-CoV and SARS-CoV [38]. In addition, a wide-spectrum anti-CoV inhibitor, N3, was shown to inhibit the proteolytic activity of MERS-CoV 3CLpro [39]. Viral helicase and RNA-dependent RNA polymerase could potentially be targeted to block MERS-CoV replication, and this approach would also be useful in treating other human CoV infections [30]. One study suggested the use of antisense oligonucleotides targeting Smad7 and small molecule inhibitors of FGF2 receptor tyrosine kinase to suppress MERS-CoV induced apoptosis and replication [29].

Accurately diagnosing MERS-CoV and other viral infections is a critical step prior to prescribing treatment to patients. A new test, called ViroCap, has been designed to diagnose viruses from 34 different families [40]. ViroCap can test for all the viruses at the same time, check for variant strains of the same virus, and is almost as sensitive as the polymerase chain reaction (PCR). Future studies should attempt to validate the accuracy of Virocap in diagnosing infectious viral agents in larger trials (Fig. 4).

FIG. 4 Future directions for MERS-CoV research: This figure depicts the future directions for MERS-CoV research. It is important to investigate the biochemical pathways hijacked by MERS-CoV, DPP4 polymorphisms and MERS-CoV disease, therapeutic potential of sDPP4 in MERS-CoV patients, and the side effects associated with proposed DAAs and IAAs. The development of a universal genomics diagnostic test and looking into emerging betacoronaviruses in bats are vital in preventing future outbreaks.

SUMMARY AND FUTURE DIRECTIONS

MERS-CoV is an emerging betacoronavirus that is associated with a high mortality rate and can be transmitted via respiratory secretions. Therefore, it is critical to understand how viral and host factors interact with each other to allow the virus to efficiently infect and...
replicate in humans. This article explores the different viral and host factors associated with MERS-CoV transmission and pathogenesis, and how this knowledge can aid in the development of therapeutics. MERS-CoV transmission is facilitated by the interaction between viral S1 RBD and host receptor DPP4 [15, 16]. Furthermore, MERS-CoV can utilize multiple host proteases to cleave the S protein, which facilitates membrane fusion. MERS-CoV has been shown to primarily infect cells in the lower respiratory tract of humans, but it can also infect immune and kidney cells [10, 29]. An overactive inflammatory response, induction of apoptosis, and cytokine dysregulation are vital factors that contribute to the high pathogenicity of MERS-CoV. Future treatments for MERS-CoV can inhibit virus entry, fusion, polyprotein cleavage, and replication by targeting both viral and host factors. Human pathogenic viruses are continually emerging from zoonotic sources due to the frequent mixing of different animal species in densely populated areas. Within the last fifteen to twenty years, numerous zoonotic enveloped viruses such as Ebola, SARS-CoV, and MERS-CoV have caused major outbreaks in human populations. Thus, to avoid future outbreaks, it is essential to develop a universal genomics diagnostic test which can distinguish between all the different types of viruses (Fig. 4). This will allow physicians to make the correct diagnosis, implement effective safety protocols, and provide patients with the appropriate treatment. It is critical to look at the side effects associated with the different DAAs and IAAs that have been proposed for MERS-CoV treatment. The use of soluble DPP4 to treat MERS-CoV infected patients needs to be investigated in future studies. Elucidating the host biochemical pathways hijacked by MERS-CoV can provide the missing link between co-morbidities and higher mortality rates in infected patients. The effect of DPP4 polymorphisms on MERS-CoV transmission and pathogenesis needs to be explored. Finally, it is important to investigate emerging, potentially pathogenic betacoronaviruses from bats to help predict the next CoV outbreak.

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