Chapter 12
Coronavirus and the Cytoskeleton of Virus-Infected Cells

Yifan Xing, Qian Zhang, and Yaming Jiu

Abstract The cytoskeleton, which includes actin filaments, microtubules, and intermediate filaments, is one of the most important networks in the cell and undertakes many fundamental life activities. Among them, actin filaments are mainly responsible for maintaining cell shape and mediating cell movement, microtubules are in charge of coordinating all cargo transport within the cell, and intermediate filaments are mainly thought to guard against external mechanical pressure. In addition to this, cytoskeleton networks are also found to play an essential role in multiple viral infections. Due to the COVID-19 epidemic, including SARS-CoV-2, SARS-CoV and MERS-CoV, so many variants have caused wide public concern, that any virus infection can potentially bring great harm to human beings and society. Therefore, it is of great importance to study coronavirus infection and develop antiviral drugs and vaccines. In this chapter, we summarize in detail how

Yifan Xing and Qian Zhang contributed equally with all other contributors.

Y. Xing
Shanghai Institute of Immunity and Infection (Formerly Institut Pasteur of Shanghai), Chinese Academy of Sciences, Shanghai, China

University of Chinese Academy of Sciences, Beijing, China
e-mail: yfxing@siii.cas.cn

Q. Zhang
Unit of Cell Biology and Imaging Study of Pathogen Host Interaction, The Center for Microbes, Development and Health, Key Laboratory of Molecular Virology and Immunology, Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, China
e-mail: qzhang@siii.cas.cn

Y. Jiu (✉)
Shanghai Institute of Immunity and Infection (Formerly Institut Pasteur of Shanghai), Chinese Academy of Sciences, Shanghai, China

University of Chinese Academy of Sciences, Beijing, China

Unit of Cell Biology and Imaging Study of Pathogen Host Interaction, The Center for Microbes, Development and Health, Key Laboratory of Molecular Virology and Immunology, Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, China
e-mail: ymjiu@siii.cas.cn

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the cytoskeleton responds and participates in coronavirus infection by analyzing the possibility of the cytoskeleton and its related proteins as antiviral targets, thereby providing ideas for finding more effective treatments.

Keywords Cytoskeleton · Coronavirus · Infection · SARS-CoV-2 · Actin · Microtubule · Intermediate filaments

Introduction to the Cytoskeleton

Cytoskeleton

The cytoskeleton constitutes a filamentous network in eukaryotic cells, which consists of actin filaments, microtubules, and intermediate filaments. Cytoskeleton not only functions in cell structure and behavior regulation including cell morphogenesis, epithelial–mesenchymal transition (EMT), and cell migration but also participates in important intracellular life activities including protein and virus replication and transport (Wang et al. 2021), vesicle trafficking (Shi et al. 2020), autophagy (Helfand et al. 2011), and so forth. Over the years, extensive studies have reported that many viruses hijack cytoskeleton networks to complete their own propaganda (Foo et al. 2015; Denes et al. 2018; Miranda-Saksena et al. 2018; Bedi et al. 2019; Zhang et al. 2019), showing the importance of this "cell scaffolding" to viral infection. In this chapter, we will introduce the relationship between host cytoskeleton and coronaviral infection, making a systematic summary of cytoskeleton-related pathogen–host interaction.

Actin Filaments

Actin filaments, also known as microfilaments, are on average ~ 7 nm in diameter. They are mainly composed of multimers assembled from globular (G) actin and other components including actin-binding protein, myosin, tropomyosin, and α-actinin. On the one hand, the common biological functions of actin filaments are regulating cell attachment, spreading, motility, endocytosis, cell division, and the control of cytoplasmic circulation. Actin filaments can be aggregated into bundles to form stress fibers and are fixed to the extracellular matrix through focal adhesions, thereby providing shear resistance to cells. The dendritic structure formed by actin filaments supports the shape of villi on the surface of epithelial cells in the respiratory tract and digestive tract. However, because it does not contain myosin or tropomyosin, here it possesses no contractility. The actin filament backbone is highly dynamic. The formation of lamellipodia when aggregation occurs can regulate cell deformation and movement. Unassembled G-actin and fibrillar actin

(F-actin) are assembled and disassembled in a dynamic pattern to promote cell movement.

On the other hand, actin filaments are also involved in the pathological processes of cells, such as cell chemotaxis, adhesion, phagocytosis (May et al. 2001), T-lymphocyte activation (Billadeau et al. 2007), and so forth. For nerve cells, actin shows neuronal polarity and is essential for signal transduction and synaptic structure stability (Xu et al. 2013). In addition, increasing studies have reported that the actin filament system provides the driving force required for virus assembly (Wang et al. 2021), budding and releasing after virus infection, thereby participating in the regulation of the virus life cycle. In a study of the interaction of the rabies virus (RABV) multifunctional matrix protein (M) with the cytoskeleton, the authors revealed actin as a binding partner of the M protein, which may regulate the viral assembly and budding in the pathogenesis of rabies (Zandi et al. 2021). Another study on DENV infection found that viral infection significantly increased cell motility and disruption of the actin filaments (Jhan et al. 2017). There are also studies on IPEC-J2 cells showing that infected with porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) affect the remodeling of actin filaments. Drugs that inhibit virus replication and release can act as anti-virus targets to regulate the remodeling of microfilaments (Zhao et al. 2014). The above findings demonstrate the significant involvement of the actin filaments system in virus infection-related diseases.

Microtubules

Microtubules, as the backbone of the cytoskeleton, are composed of the ~55 kD α- and β-tubulin, with a diameter of ~25 nm. The usually found tubular forms are single microtubules, duplex microtubules, and triplet microtubules. The most common form exists as tubulin (αβ) dimers and multimers in a head-to-tail manner to form tubulin fibrils. The process of heterodimerization of α-tubulin and β-tubulin is dynamically reversible. Many cellular structures are known to be composed of microtubules, including cilia, flagella (which contribute to motility), and mitotic spindles (which control spindle production and motility during cell division). In addition, microtubules also possess kinetic properties of polymerization and depolymerization and can form the cytoskeleton together with other fibers to maintain cell morphology. Microtubules that have been reported so far play an important role in the processes of cell proliferation and division, signal and material transduction, and the localization and function of organelles depend on the stability of these structures.

Microtubule-associated proteins (MAPs) can promote the aggregation and stability of microtubules and are essential components of microtubule structure and function. Microtubule proteins undergo various posttranslational modifications, including detyrosinylation, acetylation, polyglutamylation, and polyglycinylation, which are also reversible. These modifications play a key role in proteolysis, signal transduction, gene expression regulation and protein interactions. Studies have

shown that the knockdown of microtubule-associated protein 1S (MAP1S) also causes autophagy defects and promotes hepatocellular carcinoma. Overexpressed Tau (MAPT) impedes the transport of synaptic vesicles and organelles in vivo, and its reduction rescues the defect in axonal transport in a mouse model of Alzheimer's disease (Ukmar-Godec et al. 2020). Although the microtubule itself does not produce contractile force, it can induce the assembly and disassembly of actin stress fibers and focal adhesions by activating RhoA and then stimulating the downstream effector ROCK (Ezratty et al. 2005), to depolymerize and induce actin stress fiber formation and cell shrinkage.

Viruses often rely on an intact microtubule network during multiple stages of the replication cycle. Some viruses associate directly with microtubule-dependent motors for the transport of intact virions, capsids, individual viral proteins, or RNA, to sites of replication and plasma membranes (Wang et al. 2018). Viral particles move along microtubules within the cell, and the long-range transport speed can reach a very high level. It has been demonstrated that members of the Herpesviridae (Sodeik et al. 1997), Adenoviridae (Suomalainen et al. 1999), Parvoviridae (Suikkanen et al. 2003), Poxviridae and Baculoviridae utilize microtubules and the actin cytoskeleton for cytoplasmic transport or transport in the cytoplasm (Döhner and Sodeik 2005). During virion assembly and release, microtubules are also used to transport within extracellular vesicles or to transport capsids and nucleoprotein granules from the cytoplasm out of the budding compartment. In conclusion, the microtubule system plays a critical role in the transport of intracellular 'cargoes', cell shape, polarity, and motility. In the context of viral infection, the microtubule cytoskeleton can be hijacked by viruses for their directional transport to support their infection.

Intermediate Filaments

Intermediate filaments, ~10 nm in diameter, vary widely in composition in different cells, including vimentin, keratin, desmin, neuronal fibers, and neuroglial filaments. Intermediate filaments display tissue-specific expression, such as 'acidic' keratins are specifically expressed in epithelial tissue, and desmin is expressed in smooth muscle, neurofilaments in the nervous system, etc. In terms of structure, they are arranged in opposite directions to each other during the assembly process and thus show no polarity. Nevertheless, they have stronger elasticity and can withstand higher mechanical pressure, which makes the intermediate filaments the most stable cytoskeletal system. Of note, vimentin is the most abundant component in the intermediate filament protein family, responsible for maintaining the integrity of the cell shape against external mechanical pressure.

Early research on intermediate filaments mainly focused on the aspect of mechanical support. But, with the progression of research, it was found that there was also a hub that regulated many signaling pathways. For instance, studies indicated that vimentin is involved in signaling pathways such as Raf-1 and RhoA and regulates

TNF-α mediated apoptosis. Moreover, vimentin also can bind to secretagogues to induce apoptosis in different types of cancer cells. These studies suggest that vimentin can be used as a potential target for antitumor therapy. The intermediate filaments are also involved in the regulation of 14–3-3 and mTOR signaling pathways. The deletion of keratin 17 can cause the 14–3-3 protein to fail to aggregate in the cytoplasm, thereby activating the mTOR signaling pathway and causing cell morphological changes. In addition, intermediate fibers also affect many signaling pathways, such as PKC, PKA, JNK, CaMK II, Akt, and phosphatase pathways.

In recent years, more studies have focused on the regulatory mechanism of the intermediate filament vimentin in viral infection. For example, vimentin affects pathogen invasion as a receptor and is involved in transcellular migration and immune responses (Döhner and Sodeik 2005). Another study found that surface vimentin in Neuro-2a cells interacts with the Chandipura virus (CHPV) and acts as a receptor to promote the binding of CHPV to cells, which in turn affects the replication process after virus entry (Döhner and Sodeik 2005). In addition to the regulation of virus entry and replication, vimentin also affects the release of the virus from the cell. Polly Roy's team (Bhattacharya et al. 2007) found that disruption of vimentin structure resulted in an increase in cell-associated bluetongue virus (BTV) and a decrease in the amount of virus released by infected cells by demonstrating that the association of BTV particles with intermediate filaments is driven by the interaction of VP2 with vimentin to promote viral efflux.

In summary, although early researchers identified the importance of cytoskeletal networks for viral infection, the mechanisms by which viruses invade host cytoskeleton, regulators, and dynein adapters have received particular attention due to the current worldwide mass COVID-19 infection. Future studies, especially the study of host signaling pathways and downstream effector mechanisms, will undoubtedly provide important new ideas for the underlying mechanisms by which the cytoskeletal system functions during viral infection.

Coronavirus

The Structure and Life Cycle of Coronavirus

The coronaviruses belong to the genus coronavirus in the family Coronaviridae of the order Nidovirales in the systematic taxonomy. The shape is spherical (~125 nm in diameter), and the surface is covered with stick-shaped spikes (S protein), showing the appearance of a corona, with a diameter of about 80–120 nm (Snijder et al. 2003; Fehr et al. 2015). In terms of structure, coronaviruses are enveloped viruses, and virus particles are composed of two parts, the outer envelope and the inner helical nucleocapsid. Its genome is about 27 ~ 32 kb in length, and it is a linear single-stranded positive RNA chain with a methylated cap-like structure at the 5′ end and a poly(A) tail at the 3′ end.

Coronaviruses are widespread and can infect humans, birds, and mammals including bats, felines, rodents, and pigs (Cui et al. 2019). Coronaviruses are divided into four genera (α, β, γ, and δ), and there are currently seven known coronaviruses that can infect human (Carod-Artal 2020). Human coronaviruses (HCoV) are divided into α-CoV and β-CoV (MERS-CoV, SARS-CoV, HCoVOC43, and so forth) (Malik 2020). Since various physiological functions of viruses are determined by proteins, people focus more on the proteins encoded by the coronavirus genome. The main structural proteins that regulate the assembly and are encoded in all coronavirus genomes are: the spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) (Brian et al. 2005), and the genes encoded all appear in 5′ to 3′ order.

The coronavirus protruding surface densely glycosylated spike (S) protein binds to the host cell surface receptor angiotensin-converting enzyme 2 (ACE2) through plasma membrane fusion, phagocytosis, micropinocytosis, and clathrin-mediated or clathrin-independent endocytosis into infected cells. The protein sequence of the S1 domain of the virus is not conserved, and there are great differences between different coronavirus species, which limits the binding of the virus to the host cell surface receptor at the early stage of infection. Therefore, the type of coronavirus S protein receptor determines the species and tissue sensitivity of coronavirus. Upon entry into the host cell, the multiprotein translation process is activated, resulting in the production of smaller proteins that form a series of nonstructural proteins of the viral transcriptase-replicase complex. At the same time after entering the cell, the virion genome RNA is released into the cytoplasm, translated, replicated, and the viral replicase and other complexes are assembled. First, the positive-strand RNA is translated to generate the negative-strand RNA polymerase precursor protein, and then RNA polymerase is generated under the hydrolysis of the protein to generate the antisense negative-strand template. Next, subgenomic mRNAs are synthesized from the minus-strand subgenomic template, whose posttranslational products are the structural proteins of the virus. Following replication and subgenomic RNA synthesis, encapsulation occurs, leading to the formation of mature viruses, a process that takes place in the endoplasmic reticulum and Golgi apparatus. After assembly, virions are transported to the cell surface through vesicles and fused with the plasma membrane through exocytosis, and are then released into a new extracellular environment to infect other host cells (Cui et al. 2019; Malik 2020).

SARS-CoV-2 and COVID-19 Infection

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been around since 2019, causing a disease called coronavirus disease 2019 (COVID-19). The virus spread rapidly, indeed spreading throughout the world in a short period, and maintained high contagiousness and continued outbreaks in countries around the world. As of June 2022, 529 million cases and 6.29 million deaths have been

reported. SARS-CoV-2 is a large positive-stranded RNA genome belonging to the family Coronaviridae and is a β-CoV.

Studies have shown that the S protein plays an important role in SARS-CoV-2 evolution and spread, but so far, various genes that mainly encode S protein elements are frequently mutated (Hatmal et al. 2020). This has led directly to the creation of new, more aggressive mutants including Omicron, Delta, and alpha mutants since the discovery of the novel pathogenic coronavirus SARS-CoV-2 in 2019. The emergence and spread of these mutant strains have created new and ongoing challenges around the world. In another study, the authors demonstrated that the SARS-CoV-2 N protein promotes NLRP3 inflammasome activation to induce excessive inflammation. The N protein promotes the maturation of proinflammatory cytokines and is observed at the cellular level in vitro and in vivo in mice. More importantly, the N protein aggravated lung injury and accelerated death in mouse models of sepsis and acute inflammation (Pan et al. 2021). At present, research teams have emphasised the overproduction of inflammatory cytokines and chemokines in COVID-19 patients, including IL-6, IL-8, IL-1β, TNF-α, IFN-γ, MIP1α and 1β, CCL2, CCL5, CCL20, CXCL1, CXCL2, CXCL8, CXCL10, and CXCL17(X. Ren et al. 2021; Del Valle et al. 2020; Jamal et al. 2021; Xu et al. 2020). Moreover, SARS-CoV-2 has been reported to cause long-term respiratory and neurological sequelae in addition to inflammatory responses leading to lung damage (Wang et al. 2020a).

Hazards of Other Virus Infections

We know that human-to-human transmission is primarily through respiratory droplet contact, but viruses are not always confined to the respiratory tract, liver and gut, and in some cases, they can invade the central nervous system and cause neurological systemic disease (Carod-Artal 2020). Examples include human coronaviruses (OC-43, 229E, MERS, and SARS) and some animal coronaviruses (porcine hemagglutinating encephalomyelitis coronavirus). Neurological symptoms such as headache, dizziness, myalgia, and loss of smell have been reported in patients affected by the coronavirus, as well as with encephalopathy, encephalitis, necrotizing hemorrhagic encephalopathy, stroke, seizures, rhabdomyolysis, Guillain–Barre syndrome, etc.

Here, this review will place more emphasis on the mechanism of action between SARS-CoV-2 and the host cytoskeleton, providing possibilities for mankind to conquer coronavirus-like viruses.

Response of the Host Cell Cytoskeleton Network Following Coronavirus Infection

Viral infections alter cell morphology, as well as host gene expression and protein posttranslational modification. Like most positive-strand RNA viruses (Paul et al. 2013; den Boon et al. 2010; Zhang et al. 2019), coronaviruses infection causes visible remodeling of the morphology of cellular membranes, subcellular organelles, and cytoskeleton network (Snijder et al. 2006; Wen et al. 2020). Of note, the cytoskeleton, the most basic scaffold of cells, is involved in all aspects of cell morphological remodeling.

Coronavirus Infection Induces Expression Level or Posttranslational Modification Changes of the Cytoskeleton and its Related Proteins

Numerous studies have indicated that coronavirus infection induces rapid changes to the host cytoskeleton network, by both omics analysis and bioinformatics predictive analysis. For instance, the phosphorylation level of several cytoskeleton proteins, such as vimentin (Ser39 and Ser56), stathmin (STMN1 Ser16 and Ser25), and α-catenin (CTNNA1 Ser641), was found to be regulated during SARS-CoV-2 infection (Bouhaddou et al. 2020). Furthermore, it has been predicted that the nonstructural protein 5 (NSP5) and 3C-like (3CL) protease of SARS-CoV-2 could cleave microtubule organization-associated proteins and cytoskeleton proteins, respectively (Scott et al. 2022; Chen et al. 2022). Moreover, the expression level of cytoskeleton-related proteins also showed marked changes after infectious bronchitis virus (IBV) and TGEV infection (Emmott et al. 2010; Cao et al. 2012; Cao et al. 2011; Zhang et al. 2013). Additionally, not only for mRNA, microarray analysis revealed the changes in noncoding RNA and found that miRNA target genes are associated with actin cytoskeleton regulation after IBV stimulation (Lin et al. 2019). Expression of individual viral proteins, such as N protein and nonstructural protein 7 (NS7) of Porcine deltacoronavirus (PDCoV) in cells also results in changes in the expression level of cytoskeleton-associated proteins including ezrin, α-actinin-4 and tubulin (Lee et al. 2015; Choi et al. 2019). In addition to infection-induced intracellular changes, cytoskeleton-associated proteins were also found to be incorporated into assembled virions. Using mass spectrometry analysis, actin, tubulin, vimentin, and many cytoskeleton binding proteins such as annexin A2 and destrin were all identified in association with the IBV virion (Dent et al. 2015; Kong et al. 2010). These findings provide strong supports that cytoskeletal proteins are closely associated with coronavirus infection.

As the pathogen causing the global COVID-19 pandemic, SARS-CoV-2 infection is highly cytopathic, which calls for more sophisticated insights into how this virus alters host cells. These changes are thought to create a more suitable

environment conducive to the viral life cycle, despite inducing substantial cell perturbation and eventually causing cell death. One study provided a comprehensive repository of SARS-CoV-2-induced ultrastructural cell changes (Cortese et al. 2020). Specifically, they analyzed the alterations of three types of cytoskeleton network, actin filaments, microtubules, and intermediate filaments in SARS-CoV-2-infected A549-ACE2 cells. An accumulation of cortical actin at the plasma membrane was observed in infected cells. Moreover, actin in the cytoplasm was also found to wrap around the SARS-CoV-2 spike protein to form a ring-shaped signal, suggesting that actin may be involved in the viral life cycle. In addition, vimentin intermediate filaments were shown to form a cage-like structure in the perinuclear region that incorporated double-stranded RNA (dsRNA). Microtubules are located at the periphery of this 'cage' and have no spatial overlap with viral dsRNA. dsRNA is presumed to be a viral replication intermediate and located at the interface between the viral replication site and double-membrane vesicles (DMVs) (Klein et al. 2020; Snijder et al. 2020). Therefore, vimentin intermediate filaments might serve as a scaffold or boundary to compartmentalize viral replication organelles from others. In order to record the dynamics of vimentin cage formation, the authors performed live cell imaging and found that the majority of cage formation events were detected between 6 and 9 hours after infection, with time leading to massive cell death (Cortese et al. 2020). These findings provided compelling evidence that SARS-CoV-2 infection alters the cytoskeleton network, which potentially reflects an impact on the viral life cycle or virus-induced cell death (Fig. 12.1). During infection, all three types of cytoskeleton networks showed significant changes. Below, we summarized how coronavirus utilized and modified the cytoskeleton network during infection, in relation to actin filaments, microtubules, and intermediate filaments.

Coronavirus Infection Induces Actin Filament Rearrangement

The most visible changes caused by coronavirus infection occur in the cell membrane region, which is mainly associated with actin filament remodeling. Using high-resolution scanning electron microscopy (SEM) and transmission electron microscopy (TEM), structural changes on the cell surface can be readily captured. Clearly, a ruffled host cell and thick-ended edge appeared after SARS-CoV-2 infection (Caldas et al. 2020). A similar phenomenon also occurs in SARS-CoV-infected Vero cells (Ng et al. 2004). These changes are considered to be more conducive to the early step of infection, especially adhesion of the virion particles to cells. Likewise, researchers also observed an increased cell surface projection in SARS-CoV-2 infected cells coexisting with SARS-CoV infection, which may facilitate the release of progeny virus as a result of the driving force offered by actin filaments (Caldas et al. 2020; Ng et al. 2004; Bouhaddou et al. 2020) (Fig. 12.2b). Moreover, a thin (<0.7 μm) strand of F-actin containing tunneling nanotubes (TNT) appeared between two cells in the case of SARS-CoV-2 infection, which may provide molecular information transfer

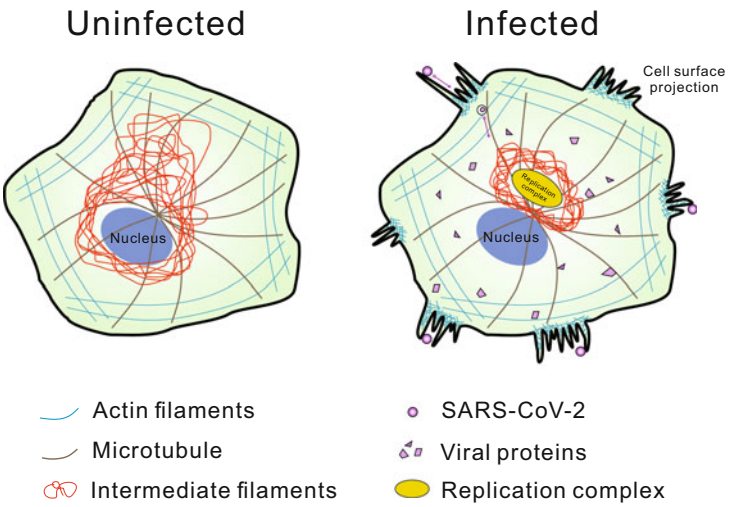

Fig. 12.1 Changes of cytoskeleton network upon SARS-CoV-2 infection. In normal cells, stress fibers exist under the cell cortex to support cell shape. Cells with smooth edges and almost no protrusions (LHS). Microtubules are mainly responsible for the transport of cargos in cells to maintain normal life activities. Intermediated filaments vimentin is distributed in the cytoplasm, with aggregation near the nucleus. Upon SARS-CoV-2 infection (RHS), many cell surface projections stretch out, mediating virus entry or providing force for the release of progeny virus. Actin filaments are clearly rearranged in these projection areas. Meanwhile, microtubules are hijacked by viruses to transport viral proteins or virus-containing vesicles to complete their own life cycle. For intermediate filaments vimentin, the infection makes these filaments shrink in the perinuclear region, with a viral replication component (encircled), which may provide a location for SARS-CoV-2 to effectively replicate

as well as viral cell-to-cell transmission (Caldas et al. 2020). In addition, a study indicated that there is no need for direct viral infection, only a single viral protein, such as SARS-CoV nucleocapsid (N) protein, is capable of inducing p38 mitogen-activated protein kinase (MAPK) downstream actin reorganization (Surjit et al. 2004).

As for SARS-CoV-2 and SARS-CoV, the rearrangement of actin cytoskeleton is common in many other coronavirus infections. Porcine hemagglutinating encephalomyelitis virus (PHEV), transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhea virus (PEDV) infection cause acute enteritis in swine of all ages with high mortality in piglets (Chae et al. 2000). It has been reported that PHEV, TGEV and PEDV infection leads to active actin rearrangement (Li et al. 2017; Lv et al. 2019; Zhao et al. 2014; Sun et al. 2017). In order to test whether the internalization of virions is accompanied by actin remodeling, F-actin and DiD-PHEV were fixed and stained at different time points after infection in Neuro-2a cells. DiD-PHEVs were found to be associated with filopodia protrusions at the cell surface initially, then the bound viruses surf toward the foot of filopodia with actin retrograde flow. This process is accompanied by actin stress fiber depolymerization, resulting in a more rounded cell shape. Lastly, actin accumulated in

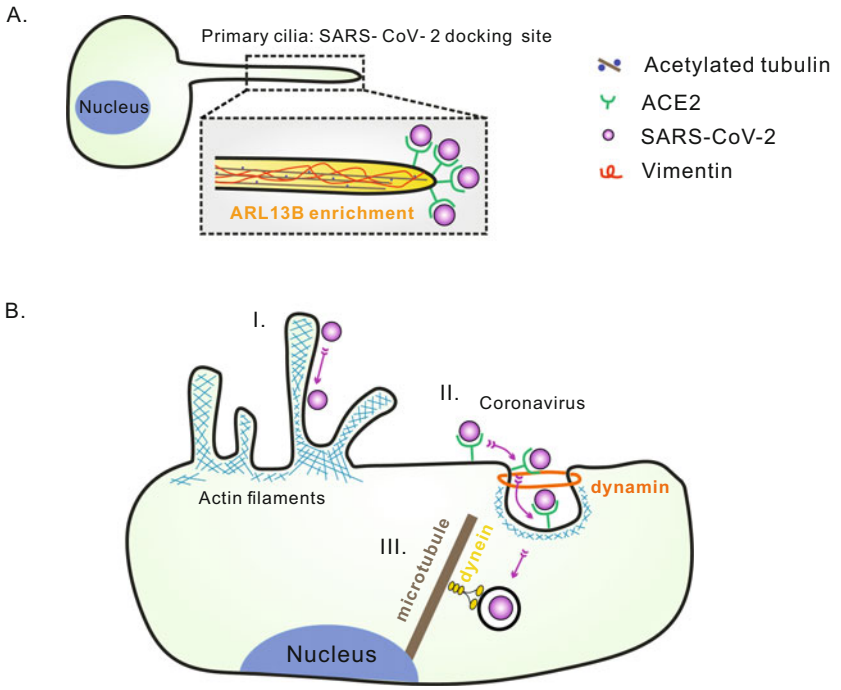

Fig. 12.2 Cytoskeleton-related internalization mechanism of coronavirus. (**a**) Primary cilia serve as the SARS-CoV-2 docking site: vimentin, SARS-CoV-2 spike protein, and ACE2 are found to colocalized in ARL13B enrichment primary cilia structure. The colocalization of these three proteins is thought to cooperate together to mediate virus docking and leads to ciliary dysfunction, thus initiating infection (Lalioti et al. 2022). (**b**) Invasion model of coronavirus: Firstly, coronavirus usually binds to the cell surface projections and then surfs to the cell body. Actin filament rearrangement significantly changes below the cell membrane in these regions (Caldas et al. 2020; Ng et al. 2004; Bouhaddou et al. 2020). Secondly, the virus specifically binds with receptor ACE2, mediating the endocytosis process. The endocytosis of coronavirus usually is clathrin-dependent, and the segment of virus-containing vesicles with the plasma membrane is dependent on microtubule-associated protein dynamin (Van Hamme et al. 2008; Owczarek et al. 2018; Wang et al. 2020b; Milewska et al. 2018). Lastly, virus-containing vesicles move along microtubules to move into the cell, this process usually relies on the motor protein dynein (Hou et al. 2019; Hagemeijer et al. 2010; Pasick et al. 1994; Kalicharran et al. 1995)

flaky pseudopods after virions were transported to the cell body. This process gives us a whole picture of how the actin cytoskeleton flow movement occurs during PHEV uptake at a very early stage (Li et al. 2017). At the molecular level, PHEV infection stimulates the integrin α5β1-FAK (focal adhesion kinase)-Rac1 (Ras-related C3 botulinum toxin substrate 1)/Cdc42 (cell division cycle 42)-PAK (p21-activated protein kinases)-LIMK (LIM kinase) axis, resulting in the dynamic of cofilin activity and F-actin rearrangement (Lv et al. 2019). Similarly, TGEV actives phosphoinositide-3 kinase (PI3K) through viral spike protein binding to epidermal

growth factor receptor (EGFR), leading to the activation of cofilin and F-actin reorganization by Rac1/Cdc42 GTPases (Hu et al. 2016).

Microtubules Are Hijacked by Coronavirus for Trafficking

Microtubules are important for intracellular cargo transport, relying on the motor proteins dynein and kinesin (Hirokawa 1998; Welte 2004; Ross et al. 2008). In the context of viral infection, microtubules are usually employed by viruses to transport their own components or host factors which are beneficial to the viral life cycle (Döhner et al. 2005; Naghavi et al. 2017). To visualize the intracellular viral movement, the porcine epidemic diarrhea virus (PEDV) was successfully labeled by quantum dots without affecting its growth kinetics. The microtubule-dependent movements were classified into three types according to the localization in cells (near-cell membrane (CM) region, middle-cell cytoplasm (CC) region, and near-microtubule organizing center (MTOC) region) with different moving characteristics by live cell tracking (Hou et al. 2019). Similarly, in infected cells with recombinant nsp2-GFP tagged mouse hepatitis coronavirus (MHV), the GFP-positive foci were also associated with or in close proximity to the microtubules. Live cell imaging futher revealed that the movement of these foci is microtubule-dependent (Hagemeijer et al. 2010). Moreover, the nucleocapsid protein of JHM virus (JHMV), a neurotropic murine coronavirus, was found to interact with microtubule-associated protein tau, and the trafficking of JHMV protein was also dependent on microtubules (Pasick et al. 1994; Kalicharran et al. 1995) (Fig. 12.2b).

Detection of Intermediate Filament Changes upon Infection

Despite much knowledge on the different types of intermediate filament proteins in cells, such as vimentin, nestin, lamin, etc., studies on intermediate filament proteins during coronavirus infection are limited. Only one previous report showed vimentin network rearrangement upon SARS-CoV-2 infection, which may contribute to its replication (Cortese et al. 2020). Besides, there are also some omics analyses indicating that the expression level of intermediate filaments proteins changes during coronavirus stimulation. Therefore, the effect of viral infection on intermediate filaments should receive more extensive attention and research.

In the following section, we summarize the effect of coronavirus infection on the host cytoskeleton network, showing a close interaction between the two. Briefly, actin filaments are the most significantly changed cytoskeleton element, with more protrusions formed on the cell surface, which are considered to be beneficial to viral entry and release. Microtubules are always hijacked by viruses to transport their own proteins and host factors for propaganda. For intermediate filaments, more attention

should be paid during coronavirus infection. Next, we will consider how the cytoskeleton participates in the viral life cycle.

Host Cytoskeleton Participates in Coronavirus Life Cycle

As we mentioned above, coronaviruses cause drastic cytoskeletal morphological changes, indicating that the cytoskeleton network plays an essential role in the process of infection. Here we summarize how the cytoskeleton and its related proteins in host cells regulate the coronaviral life cycle at different steps of infection.

Host Cytoskeleton Protein Facilitates Coronaviral Internalization

Various compounds that alter the cytoskeleton network integrity and dynamics have been used to investigate the contribution of the cytoskeletal network to SARS-CoV-2 infection. In a recent study, Vero E6 cells were treated with Latrunculin A (pan actin filament disrupting drug) 2 hours postinfection. Surprisingly, although the actin network showed obvious changes upon coronavirus infection, prior disruption of the actin network did not show any apparent difference in viral dsRNA and extracellular infectivity (Cortese et al. 2020). However, another work showed an inhibition of the entry of SARS-CoV-2 pseudoviral particles into HEK293-ACE2-GFP cells, when cells were treated with the same Latrunculin A (Zhang et al. 2020). These two studies leading to contradictory results illustrate a problem in that the dosing time is critical, which may cause different consequences. To test the effect of drugs on virus invasion, adding drugs before or at the same time with the virus is much better than postinfection treatment. Consist with this idea, a study used four actin network-altering drugs, Latrunculin A, Jasplakinolide, Cytochalasin B, Cytochalasin D, and one microtubule-altering drug nocodazole, to identify the role of cytoskeleton during MHV infection. These workers found that only when they added all the drugs at an early time, rather than at a late stage of infection was MHV infection reduced, revealing the importance of the actin and tubulin network in the entry step of infection (Burkard et al. 2014).

Not only for SARS-CoV-2, but drugs interfering with actin networks also seem harmful to many other coronavirus infection establishments. For instance, Jasplakinolide (stabilization of actin cortex) treatment inhibits the entry of HCoV-OC43 (Owczarek et al. 2018). For the invasion of HCoV-NL63, the two actin inhibitors Cytochalasin D (inhibits actin polymerization) and Jasplakinolide (binds F-actin and stabilizes actin filaments) both block viral particles from penetrating the cell, while the microtubule interfering drug nocodazole shows no effect on viral entry (Milewska et al. 2018). Infection with infectious bronchitis virus (IBV) also

causes actin network rearrangement as we mentioned above. However, a study indicated that the entry of IBV can be enhanced by Cytochalasin D or Jasplakinolide (Wang et al. 2019). Nevertheless, these results are self-contradictory and out of expectation, the author cannot give an explicit explanation, indicating the complex process of viral invasion.

After attachment, the internalization of many viruses is reported to rely on dynamin, a microtubule-associated protein and usually functions by pinching off the invaginated vesicles from the cell membrane. For example, both dynamin inhibitory peptide and dominant-negative dynamin significantly inhibited the internalization of feline infectious peritonitis virus (FIPV) (Van Hamme et al. 2008). During this process, others also indicated that myosin light chain kinase (MLCK) and myosin 1 play a vital role in FIPV internalization and subsequent transportation (Dewerchin et al. 2014). Inhibition of dynamin by its inhibitor MiTMAB (interacts with the lipid binding domain of dynamin) or dynasore (noncompetitively inhibits GTPase activity of dynamin) both significantly inhibited HCoV-OC43 entry efficiency to target cells (Owczarek et al. 2018). Similarly, MiTMAB also blocks the internalization of HCoV-NL63 (Milewska et al. 2018). Either by expression of the dynamin-1 dominant-negative mutation or knocking-down by siRNA-inhibited IBV infection, revealed the import role of dynamin-1 in IBV endocytosis (Wang et al. 2019). Furthermore, the internalization of TGEV was shown to be dynamin 2-dependent (Wang et al. 2020b). These studies reveal the general role of dynamin in the process of coronavirus infection, prompting us to take this protein family into consideration when studying other related viruses (Fig. 12.2b).

SARS-CoV and SARS-CoV-2 both utilize the host cell angiotensin-converting enzyme 2 (ACE2) for internalization. It is firmly believed that viral spike protein is important for the interaction between virions and host cell receptors. Recently, many works have indicated the role of extracellular vimentin in both SARS-CoV and SARS-CoV-2 infection, especially in the entry step (Suprewicz et al. 2021; Amraei et al. 2022; Yu et al. 2016). Early on, people revealed the direct interaction between the SARS-CoV spike protein and vimentin. By gene knocking-down and antibody neutralization assay, they further confirmed the vital role of cell surface vimentin function as a putative co-receptor in the uptake of SARS-CoV virus-like particles (Yu et al. 2016). Similarly, vimentin was also shown to bind to SARS-CoV-2 spike protein. Entry assay conducted with pseudotyped SARS-CoV-2 further confirmed the role of vimentin in facilitating virus invasion (Suprewicz et al. 2022; Amraei et al. 2022). Further, a study revealed that SARS-CoV-2 spike protein, ACE2 and vimentin appear to concur at a certain cellular structure, primary cilia, indicating that primary cilia may be the docking structure for SARS-CoV-2 invasion (Lalioti et al. 2022) (Fig. 12.2a). These results indicated that extracellular vimentin is involved in the spike protein-ACE2 complex, functioning as a critical component in mediating SARS-CoV and SARS-CoV-2 internalization, and vimentin-targeting agents may have significance for clinical treatment of infection.

In addition to ACE2, it has also been reported that the entry of SARS-CoV-2 is assisted by cell surface heparan sulfate (HS). Two drugs, BNTX and Sunitinib, targeting the HS ligand α-Syn fibril, not only block the entry of both SARS-CoV and

SARS-CoV-2 pseudoviral particles but also cause actin cytoskeleton rearrangement, revealing the essential role played by actin in the entry of virus (Zhang et al. 2020). Other proteins, such as ezrin, a membrane-actin linker, were also identified to interact with the SARS-CoV spike protein and function as a restricting host factor in the entry process (Millet et al. 2012).

Host Cytoskeleton Affects Coronaviral Replication

To confirm the role of intermediate filaments in SARS-CoV-2 infection, a study applied Withaferin A (an intermediated filaments disrupting drug) to treat cells 2 hours postinfection. As expected, there was a robust reduction in viral dsRNA and supernatant infectious virions found after 6 hours of treatment with Withaferin A. Compared with the result that vimentin intermediate filaments form a cage-like structure that incorporates double-stranded (ds) RNA, these data reveal the importance of intermediate filaments in the SARS-CoV-2 replication step (Cortese et al. 2020). Vimentin also plays a role in TGEV replication. Using siRNA to knockdown vimentin in ST (swine testis) cells, it was found that there was a significant reduction of cell-associated virus by TCID50 assay, which reflected viral replication. Meanwhile, cellular vimentin is associated with the TGEV N protein, and this interaction may help virions to transport through a functional Golgi complex for viral maturation (Zhang et al. 2015).

As for MHV, a study indicated that two stains, RSA59 and RSMHV2, showed different responsiveness to microtubules. Disrupting microtubules with colchicine or vinblastine remarkably reduced the replication of RSA59, whereas it did not affect the RSMHV2 strain, indicating the vital role of microtubule-dependent axonal transport on RSA59 infection and replication. The only difference between these two strains is the spike gene, suggesting that the microtubule-dependent transportation might be a spike protein-mediated process (Biswas et al. 2014).

Host Cytoskeleton Participates in the Coronaviral Assembly Process

A study by Wang et al. (2009) confirmed that actin interacts with infectious bronchitis virus (IBV) M protein at amino acids A159 and K160. Abolishing this interaction by A159-K160 mutation in full-length transcripts did not generate an infectious virus by electroporation using the IBV clone system, indicating that the interaction between actin and M protein is essential for progeny virus production in the late stage of infection. Moreover, Cytochalasin D treatment at the early but not late stage of replication resulted in no virion release to the supernatant, suggesting that actin may function in viral assembly and budding, but not for release.

Coronaviral Release Can Be Promoted by Cytoskeleton Proteins

To detect the role of microtubules on SARS-CoV-2 infection, Cortese et al. (2020) used nocodazole and colcemid to induce microtubule depolymerization and found that these two drugs did not affect SARS-CoV-2 infection. On the contrary, inhibiting microtubule depolymerization or polymerization by paclitaxel or vinblastine, respectively, showed a strong effect on the production of infectious extracellular viruses. This finding emphasized the comprehensive role of the microtubule network in SARS-CoV-2 infection.

In order to detect the role of actin filaments in PEDV and TGEV infection, IPEC-J2 cells were treated with Cytochalasin D or Jasplakinolide. The results showed that neither Jasplakinolide nor Cytochalasin D affected the entry of these two viruses into IPEC-J2 cells. Instead, the replication and release steps of PEDV and TGEV were notably inhibited by either Jasplakinolide or Cytochalasin D (Zhao et al. 2014). This work indicated the role of the actin network in PEDV and TGEV infection, and microtubules also play a role in TGEV infection. To be more specific, the distribution of TGEV spike protein diffused throughout the cytoplasm after nocodazole treatment, compared to the DMSO-treated group near the nucleus. Moreover, the colocalization between the TGEV spike and membrane protein was also reduced in nocodazole-treated cells. Importantly, the microtubule targeting drug nocodazole results in a reduction of infectious virions release and with less spike protein incorporated into virions (Rüdiger et al. 2016).

In this section, we summarize how the cytoskeleton and its related proteins participate in the coronavirus life cycle. In some cases, the specific affected step of infection cannot be precisely defined. To sum up, actin is mainly involved in three steps of virus infection, internalization, assembly and release. Microtubule-related proteins, especially dynamin, primarily play a role in viral internalization. The microtubule network itself is involved in coronavirus replication and release. Intermediate filament vimentin participates in viral internalization and replication. All three cytoskeleton protein networks play an essential role in coronavirus infection. It is of great significance to summarize common rules, which may enable us to understand more about coronavirus infection.

Cytoskeleton and Pathogenesis

After the SARS-CoV-2 virus enters the human body, it rapidly replicates and proliferates, blocking alveolar blood oxygen exchange and inducing a cytokine storm in the lungs. Concurrently, it interacts with neutrophils, monocytes, and immune response cells, resulting in diffuse alveolar damage (DAD), acute lung injury

(ALI), and acute respiratory distress syndrome (ARDS) (Huang et al. 2020; Jiang et al. 2020; Zhou et al. 2020), and consequently pulmonary fibrosis. These pathological manifestations are particularly severe at later stages (Li et al. 2012). It has been reported that the pathogenic mechanism of the coronavirus involves vascular permeability, airway epithelial ciliated cells, and nervous system regulation. Clarifying the pathogenic mechanism of coronavirus will help design inhibitory drugs for each key target, which is of great importance for human beings to conquer the virus. By summarizing the studies of many laboratories in recent years, we found that an increasing number of researchers have paid attention to the important role played by the cytoskeleton in the pathogenesis of this coronavirus.

The Ciliary Structure Is Involved in the Pathogenic Process

Epithelial cells lining the airways of mammals, such as nasal and pulmonary cilia, play a key role in the defense against infection (Tilley et al. 2015). Loss of cilia is one of the most striking ultrastructural abnormalities in coronavirus-infected cells. The mucociliary clearance mechanism prevents the accumulation of coronavirus particles and mucus in the lungs. It has also been reported that in human nasal epithelial cells, coronavirus infects ciliated cells and thus causes cilia shedding, inducing anosmia (Nicholls et al. 2003). Analysis of autopsy samples from patients with COVID-19 found that SARS-CoV-2 replication was predominantly in airway epithelial cells and alveoli (Hou et al. 2020). Recent studies have found that SARS-CoV-2 has a preferential tropism for ciliated epithelial cells and occasionally infects transitional and secretory cells.

The best-characterized receptor we know of SARS-CoV-2 is ACE2, and the spike-ACE2 interaction has been extensively studied. In immunoassay studies, vimentin aggregates with acetylated tubulin and ARL13B proteins in cilia-like apical structures. The overall colocalization of vimentin, spike, and ACE2 proteins was observed. The spike protein exists in the outer layer of the virus and can mediate the binding of virus particles to cellular receptors and trigger membrane fusion. Ciliated cells have been identified as one of the selective targets of SARS-CoV-2 infection in human tissue studies. Therefore, the authors speculate that vimentin plays an active role in cooperation with ACE2 in the contact between virus and cilia, which may be a specific site of virus docking and leads to ciliary dysfunction, and then mediates virus infection (Lalioti et al. 2022). Therefore, researchers have speculated that many pathological changes of COVID-19 are related to ciliary dysfunction.

Some scholars have also provided evidence from the perspective of phosphoproteomics, proving that the cytoskeleton plays a regulatory role during coronavirus infection. It was also found that kinases downstream of Rho/Rac/Cdc42 GTPases and several cytoskeletal organization-related kinases and effector proteins such as phosphorylated vimentin at site Ser39 and phosphorylated vimentin at site

Ser56 were down-regulated during infection, and cytoskeletal proteins such as motor protein myosin IIa (MYH9 S1943) were down-regulated during infection (Bouhaddou et al. 2020). Primary cilia are microtubule-based organelles (Buqaileh et al. 2021) that reside on the cell surface and sense various environmental stimuli. Analysis of the interaction between the viral nonstructural protein Nsp13 and centrosome components from the perspective of molecular biology also provides a potential molecular mechanism (Li et al. 2020a).

Other coronaviruses have also shown pathological features that target airway-ciliated cells. After the virus infects the host, ciliated cells undergo physical and chemical damage, and at the same time, the levels of microtubule cytoskeleton system-related proteins change. Including SARS-CoV and common cold coronaviruses HCoV-NL63, -OC43, -HKU1, etc. (Zhu et al. 2020; Pizzorno et al. 2020; Hao et al. 2020). Previously, Chilvers et al. (2001) inoculated HCoV 229E into the human nasal cavity, and the respiratory tract epithelium was destroyed, resulting in ciliary motility disorders; epithelial integrity was significantly lost, and the number of ciliated cells was significantly reduced, together with peripheral and central region tubulin changes. These results suggest that the microtubule cytoskeleton system is involved in the regulation mechanism of cilia and virus infection (Hou et al. 2020). In another experiment using animals as hosts, it was found that in MERS-CoV-infected dromedary camels, MERS-COV colocalized with keratin18. The experiment detected the cilia in the airways of animals by anti-α-acetylated tubulin-specific antibodies. There was extensive loss of tubulin signaling in the turbinates and trachea of infected animals (Haverkamp et al. 2018). In addition, the disease caused by Canine respiratory coronavirus (CRCoV), loss, and damage of nasal and tracheal cilia was used to assess its potential impact on the mucociliary system (Priestnall et al. 2009; Mitchell et al. 2013).

Together, these studies fully demonstrate the importance of the cytoskeleton for the regulation of ciliated cells in the pathogenesis of coronavirus, unraveling these mechanistic studies provides the basis for virus-host interactions in protective immunity, host susceptibility, and viral pathogenesis.

Lung Injury Caused by SARS-CoV-2 Is Related to Endothelial Cell Permeability Regulation

Defects in the integrity of the pulmonary vascular barrier are considered to be one of the hallmarks of pathological changes in SARS-CoV-2 infection. In general, vascular endothelial (VE) cells maintain vascular permeability at low levels (Rho et al. 2017).

However, when coronavirus infection induces inflammation, dynamic regulation of endothelial cells can limit vascular barrier function and induce immune cell extravasation, triggering the viral defense mechanism of the host. It was found that through the coiled-coil of Rho and its downstream protein kinase ROCK, nonmyosin

cytoplasm-induced myosin contraction and the formation of radial stress fibers can induce dynamic reorganization of the actin cytoskeleton (Eisenhut et al. 2020), which in turn enables Rap1 signaling in vascular endothelial cells (Yamamoto et al. 2021). This process enhances VE-cadherin-mediated cell-cell adhesion function and vascular barrier function, which can help improve the clinicopathological status of patients.

In addition to the contractile regulation of actin, the polymerization regulator of actin is also required for cytoskeletal reorganization. Rap1 inhibits Rho activity as the actin backbone regulates the adhesion function and vascular permeability of VE-cadherin. Thus, activated Cdc42/Rac1 limits vascular permeability, and the dynamic regulation of cytoskeletal organization in vascular endothelial cells and enables cells to avoid hypertonic states (Yamamoto et al. 2021).

The permeability of the lung epithelial cell barrier also plays a crucial role in SARS-CoV-2-induced lung injury and lung homeostasis. It is found that MRCKα and its downstream activation of the myosin light chain are required for the NKA β1 subunit to regulate alveolar barrier function. The synergistic effect of myosin and the actin filament cytoskeleton system can promote cell movement so that endothelial cell permeability changes dynamically with different microenvironments. In contrast, MRCKα is expressed in both the human respiratory tract and alveoli, with reduced expression in SARS-CoV-2 infected patients (Bai et al. 2021).

Relationship with the Nervous System

SARS-CoV-2 not only severely damages respiratory systems such as the trachea and lungs, at the same time neurological complications in patients with COVID-19 have also become one of the important causes of morbidity and mortality. The latest research progress points out that viral RNA appears in the brain and cerebrospinal fluid. Other evidence also illustrates the neurotropism of SARS-CoV-2 (Meinhardt et al. 2021). In the dynamic regulation of the nervous system and virus, the expression of cytoskeleton-related proteins such as fibronectin increases. This change was accompanied by elongation and contraction of the morphology of brain pericytes exposed to the S protein (DeOre et al. 2021).

The nervous system is divided into the central nervous system (CNS) (including the brain and spinal cord) and the peripheral nervous system (PNS) (including the cranial and spinal nerves). These two major parts are the dominant players in the regulation of physiological functions in the body. Clinical reports show that coronavirus can affect the central nervous system. Induction included cerebrovascular disease (Thakur et al. 2021), multifocal cerebral micro-occlusion, and stroke (Mao et al. 2020; Klok et al. 2020). Clinical symptoms include loss of smell and taste, headache, fatigue, nausea, etc. SARS-CoV-2 RNA was also shown to exist in the peripheral nervous system (Matschke et al. 2020; Schurink et al. 2020; Andalib et al. 2021). The infection causes, for example, nerve pain and skeletal muscle damage, Guillain-Barre syndrome, cranial polyneuritis, neuromuscular junction disorders,

neuro-ophthalmic disorders, and autonomic dysfunction (Andalib et al. 2021). Thus, it is also important to focus on the role of viruses in the pathogenesis of these neurological complications.

Regarding viral entry, the degree of expression of ACE2 receptors in the nervous system may affect the neurovirulence of SARS-CoV-2. This receptor is expressed in endothelial smooth muscle cells. In addition, studies have found the RNA and S protein of SARS-CoV-2 in specific cells of the brain and nasopharynx. Other investigators have also detected intact virus particles in the nasopharynx (Bilinska et al. 2020). Thus, it is proposed that the virus can infect olfactory neurons, and its proteins can enter the nervous system from the PNS terminal and the olfactory epithelium, and even exist in the medulla oblongata of the brain. In addition, other studies have proposed three pathways by which SARS-CoV-2 enters the nervous system. One is a transsynaptic pathway from nasal epithelial cells to the brain via trigeminal branches (Ferreira et al. 2020). The second is through endocytosis or exocytosis, internalization at nerve terminals, and retrograde transport. Viruses can be transported back to neuronal cell bodies along microtubules by transsynaptic transfer as well as axonal transport mechanisms (vesicular trafficking) (Zubair et al. 2020). The last is a common route for consanguineous virus entry into the nervous system, via internalization and transport across the brain endothelium, thereby crossing the intact blood-brain barrier (BBB) (Iadecola et al. 2020; Iadecola et al. 2020; DeOre et al. 2021). Recent studies have confirmed that angiotensin-converting enzyme 2 (ACE2) at the SARS-CoV-2 binding site contributes to spike-induced barrier disruption through the activation of RhoA. The authors analyzed RhoA to identify it as a key molecule regulating endothelial cytoskeleton and tight junction complex dynamics.

Neuroimmune aspects are caused by viruses. Some researchers have found that SARS-CoV-2 deregulates the vascular and immune functions of brain pericytes through the spike protein. To cope with the viral infection and maintain the homeostasis of autoimmunity, a large number of cytokines are involved. It has been reported that SARS-CoV-2 induces ACE2 downregulation in the nervous system to activate the canonical RAS pathway. Cascade events lead to oxidative stress, neuroinflammation, vasodilation, coagulopathy, and thrombosis. In addition, SARS-CoV-2 binds to toll-like receptors and releases proinflammatory cytokines such as interleukin (IL)-1, (IL)-6, which in turn induce immune responses, leading to brain tissue damage and stroke. Most COVID-19 patients also show a trend toward increased IFN release, which leads to inflammation and immune system suppression (Andalib et al. 2021). These clinical manifestations are firstly innate immune hyperactivity and then immunosuppression. This can effectively protect the body's homeostasis. Our review of the reciprocal regulation between SARS-CoV-2 and the nervous system thus summarizes new mechanistic insights into the pathobiology of cerebrovascular disease associated with COVID-19.

Possibilities of Cytoskeleton-Related Treatment of COVID-19

As of April 12th, 2022, the World Health Organization (WHO) has counted nearly 500 million confirmed cases of COVID-19 worldwide and more than six million deaths. Although global vaccination rates have reached an advanced level, the defense capacity of existing vaccines is inconclusive in the face of the continuous emergence of new virus variants (Basky et al. 2022). Numerous scholars are still exploring, aiming to determine the most effective treatment options.

As our understanding of the pandemic has grown, many different therapeutic avenues have been developed. For example, pharmacology, immunology, traditional Chinese medicine (TCM) (Ren et al. 2020), etc. At this stage, enzyme inhibitors and neutralizing antibody drugs are the main treatments for SARS-CoV-2 infection. Some clinicians have proposed anti-inflammatory agents: colchicine (Elshafei et al. 2020), as multiple sclerosis (MS) drugs: fingolimod and sipomod (Kloc et al. 2020) are currently new drugs discovered for COVID-19 treatment. Recently, many others have suggested that vimentin could serve as a new target for the treatment of COVID-19 (Li et al. 2020b; Ramos et al. 2020). It is speculated that drugs reducing vimentin expression can be used to treat patients with COVID-19.

In this review, we have paid special attention to the physiological and pathological processes of the cytoskeletal system closely related to coronaviruses such as SARS-CoV-2. Discovering the exact mechanism by which SARS-CoV-2 subverts host cells is critical for validating specific drug targets and effective treatments. In the case of actin filaments, TLRs, CLRs and other receptors (Ezrin and dipeptidyl peptidase 4) that enhance antiviral immunity and viral clearance may serve as therapeutic targets for COVID-19 (Gadanec et al. 2021). In lung disease associated with COVID-19, bradykinin and tumor necrosis factor-alpha (TNFα) disrupt the actin cytoskeleton, which could be the leading cause of death of living organisms. Ezrin peptides can be used to inhibit SARS-CoV-2 entry, as well as other viral infections, including HIV-1, hepatitis C virus, human papillomavirus, etc. (Norris et al. 2021). TNFα disrupts the human lung epithelial cytoskeleton system and exerts its effects through Rho kinase. Dedicated to restoring the integrity of the pulmonary endothelial cytoskeleton in patients with COVID-19, thereby reducing the symptoms of patients, this method deserves further exploration by researchers.

In the case of microtubules, the use of microtubule-targeted drugs to treat coronavirus-infected individuals may be effective (Norris et al. 2021). Viral load can be reduced due to targeting microtubules by its inhibitors. The approved drug vinca alkaloid causes the breakdown of the microtubule network; in contrast, paclitaxel stabilizes the microtubule system. In addition, the anti-inflammatory agent mentioned above, colchicine inhibits microtubule polymerization and has performed preliminary studies on the safety of the treatment for patients with COVID-19 (Elshafei et al. 2020).

In the case of intermediate filaments, attenuating the role of vimentin in virus-induced infection could theoretically inhibit viral infection. It has been fully

confirmed by many researchers: (1) Vimentin is a co-receptor receptor for SARS-CoV and SARS-CoV-2 entering new cells, except for ACE2; (2) Vimentin is involved in the replication of the virus life cycle (3) Vimentin exerts an anti-inflammatory effect in the cytokine storm caused by a viral infection, and causes the body's autoimmune response (Ramos et al. 2020); (4) The low expression of vimentin inhibits the epithelial–mesenchymal effect of the body transform. According to the above conclusions, it is found that the dual role of vimentin in viral infection may have synergistic advantages for patients. Therefore, some people speculate that drugs that reduce vimentin expression could be used to treat patients with COVID-19. The latest research suggests that ALD-R491 (an oral, noncytotoxic vimentin-targeting small molecule), by changing the physical properties of vimentin filaments, has preclinical efficacy against COVID-19. The authors confirmed through in vivo and in vitro experiments (Li et al. 2021) that ALD-R491 can hinder the entry and exit of viruses into and out of cells, and increase the microbicidal capacity of macrophages, thereby promoting pathogen clearance.

Summary

In this chapter, we have summarized the connection between coronavirus infection and three major cytoskeleton networks (Table 12.1). This includes the dynamic changes in the cytoskeleton and its related proteins upon viral infection, how cytoskeleton proteins regulate the coronavirus life cycle, and cytoskeleton-related pathogenesis caused by coronavirus infection. It needs to be emphasized that all three cytoskeleton networks are heavily involved in every process of infection. Firstly, the invasion of coronavirus is mainly dependent on the actin filament rearrangement. Viruses are attached to the plasma membrane and interact with its specific receptors or co-receptors, inducing actin filament-dependent filopodia formation. Endocytosis starts after the virus moves along filopodia to the cell body. These processes involved actin filaments, vimentin filaments, and several actin-related proteins. The subcellular transport of a virus or its components relies on the microtubule network. The replication of coronavirus is reported to associate with tubulin and vimentin. We know little about viral assembly. Only actin filaments have been reported to be involved in this process. Finally, the release of progeny virus also requires actin filaments and microtubules to provide the 'driving force'. Most importantly, coronavirus infection-induced pathogenesis is highly correlated with cytoskeleton networks, indicating that drugs targeting cytoskeleton proteins may have an essential influence on treating diseases caused by viral infections. Although there is a substantial increase in the understanding of the regulation of cytoskeleton components and corresponding elaborate subcellular structures in the process of coronavirus infections, there are still many questions that remain future pursuing. In particular, conducting clinical research on drugs targeting cytoskeletons may help to inspire new strategies to control infection and infection-induced pathological damage.

Table 12.1 Correlation between coronavirus infection and the cytoskeleton

No.	Virus	Involved cytoskeletal system	Modes of action	Models	Reference
1	Human coronaviruses (HCoV) OC43	Actin filaments	The virus employs caveolin-1 dependent endocytosis for the entry and the scission of virus-containing vesicles from the cell surface is dynamin-dependent. Furthermore, the vesicle internalization process requires actin cytoskeleton rearrangements.	Cell: HCT-8	Owczarek et al. (2018)
2	Human coronavirus NL63	Actin filaments	Subsequent vesicle scission by dynamin results in virus internalization, and the newly formed vesicle passes the actin cortex, which requires active cytoskeleton rearrangement.	Cell: LLC-Mk2	Milewska et al. (2018)
3	Human coronavirus 229E	Microtubules	Inoculation of healthy volunteers with human coronavirus caused disruption of the ciliated epithelium and ciliary dyskinesia. This is likely to impair mucociliary clearance.	Clinical trials	Chilvers et al. (2001)
4	Avian coronavirus infectious bronchitis virus (IBV)	Actin filaments	The actin filaments might block virion assembly and budding, but not release of the virus particles.	Cell: H1299; Vero	Wang et al. (2009)
5	Avian coronavirus infectious bronchitis virus (IBV)	Intermediate filaments; Actin filaments	Changes in intracellular mRNA levels after IBV infection by proteomic analysis. The proportions of many cytoskeletal proteins including MYO6, tubulin, vimentin, and acitn in the nucleus were changed.	Cell: Vero	Emmott et al. (2010)
6	Avian coronavirus infectious bronchitis virus (IBV)	Microtubules	Reproductive damage in hens after viral replication in the epithelium of the oviduct, resulting in loss of cilia and degeneration	Cell: Vero	Villarreal et al. (2007)

(continued)

Table 12.1 (continued)

No.	Virus	Involved cytoskeletal system	Modes of action	Models	Reference
			and necrosis of the epithelial and glandular cells.		
7	Transmissible gastroenteritis virus (TGEV)	Actin filaments	The EGFR-PI3K-Rac1/Cdc42-PAK-LIMK signaling pathway affects cofilin phosphorylation, affects actin rearrangement, and allows viruses to invade cells.	Cell: IPEC-J2; HEK293T	Hu et al. (2016)
8	Transmissible gastroenteritis virus (TGEV)	Actin filaments	TGEV can affect microfilament remodeling in IPEC-J2 cells, and drug-interfered microfilaments can inhibit virus replication and release.	Cell: IPEC-J2; ST; Vero	Zhao et al. (2014)
9	Transmissible gastroenteritis virus (TGEV)	Actin filaments; Microtubules; Intermediate filaments	Following TGEV infection, mass spectrometry analysis revealed changes in the composition of all three cytoskeletal proteins. It is speculated that changes in actin and tubulin are beneficial for virus replication.	Cell: ST	Zhang et al. (2013)
10	Porcine epidemic diarrhea virus (PEDV)	Microtubules	Microtubule, dynein, and kinesin-1 are involved in PEDV infection and can influence PEDV fusion and accumulation in the perinuclear region but cannot affect PEDV attachment or internalization.	Cell: Vero	Hou et al. (2021)
11	Porcine epidemic diarrhea virus (PEDV)	Actin filaments	Mutations in the S protein result in a higher titer. These phenomena such as CPE may be related to the rearrangement of actin.	Cell: Vero	Sun et al. (2017)
12	Porcine epidemic diarrhea virus (PEDV)	Cytoskeleton-associated protein	E-cadherin expression was decreased at the villus tip, whereas expression of N-cadherin, vimentin and snail was increased. Reduced actin expression in	Animal: Weaned pigs	Chen et al. (2021)

			microvilli and zonula occludens-1 (ZO-1). Increased protein concentration of transforming growth factor beta 1 (TGFβ1), which mediates EMT and cytoskeletal changes.		
13	Porcine hemagglutinating encephalomyelitis virus (PHEV)	Actin filaments	After infection, actin cytoskeleton depolymerization occurred, with the number of actin stress fibers decreasing, and transient blebs filled with actin forming at the cell surface.	Cell: Neuro-2a	Li et al. (2017)
14	Porcine hemagglutinating encephalomyelitis virus (PHEV)	Actin filaments	α5β1-FAK-Cofilin pathway causes cytoskeletal rearrangement.	Cell: Neuro-2a	Lv et al. (2019)
15	Porcine deltacoronavirus (PDCoV)	Microtubules	Increased N protein upregulates ezrin expression, to organize the cortical cytoskeleton by mediating interactions between actin and the plasma membrane proteins.	Cell: HEK-293 T; PAM-pCD163-N	Lee et al. (2015)
16	Porcine deltacoronavirus (PDCoV)	Microtubules	A substantial downregulation of α-actinin-4 was confirmed in NS7-expressing and PDCoV-infected cells.	Cell: HEK-293 T; HeLa	Lee et al. (2015)
17	Feline infectious peritonitis virus (FIPV)	Microtubules	Microtubule-related protein dynamin entry Dynamin inhibitory peptide and DN dynamin effectively inhibited virus internalization.	Cell: CrFK	Dewerchin et al. (2014)
18	Feline infectious peritonitis virus (FIPV)	Microtubules	Intracellular trafficking over microtubules was mediated by MLCK, myosin 1 and a small actin tail.	Cell: Cat monocytes	Van Hamme et al. (2008)
19	Mouse hepatitis coronavirus (MHV)	Microtubules	The microtubule-associated motility proteins DYNC1H1 and DYNC2H1 are important for infection with MHV (siRNA).	Cell: LR7; FCWF; HEK293T; MDCK; Vero; HeLa; LR7; HeLa-mCC1a	Burkard et al. (2014)

(continued)

Table 12.1 (continued)

No.	Virus	Involved cytoskeletal system	Modes of action	Models	Reference
20	Mouse hepatitis coronavirus (MHV)	Microtubules	Overexpression of nsp2-GFP fusion protein in MHV-infected cells and found to colocalize with tubulin.	Cell: LR7	Hagemeijer et al. (2010)
21	Mouse hepatitis coronavirus (MHV)	Microtubules	Disrupting microtubules significantly blocks neuronal transport.	Cell: Neuro2a	Biswas et al. (2014)
22	Mouse hepatitis coronavirus (MHV)	Microtubules	MHV uses microtubules (MTs) as carriers to reach the cell surface, meantime restricted MT-mediated Cx43 delivery to the cell membrane.	Cell: Astrocyte; Oligodendrocyte; C57Bl/6	Basu et al. (2017)
23	Neurotropic corona virus JHM (JHMV)	Microtubules	Specific interaction between antibodies to the microtubule-associated protein tau and the JHMV nucleocapsid protein (N).	Cell: OBL-21	Pasick et al. (1994)
24	MERS-coronavirus (MERS-CoV)	Microtubule-associated epithelial ciliary system	*Animal culture tissue*	Animal: Dromedaries	Haverkamp et al. (2018)
25	Canine respiratory coronavirus (CRCoV)	Microtubule-associated epithelial ciliary system	Reduced mRNA levels of the proinflammatory cytokines TNF-α and IL-6 and the chemokine IL-8 within 72 hours of vaccination.	In vitro organ culture system	Mitchell et al. (2013)

References

Amraei R et al (2022) Extracellular vimentin is an attachment factor that facilitates SARS-CoV-2 entry into human endothelial cells. Proc Natl Acad Sci U S A 119(6):e2113874119

Andalib S et al (2021) Peripheral nervous system manifestations associated with COVID-19. Curr Neurol Neurosci Rep 21:9

Bai H et al (2021) The Na+, K+-ATPase β1 subunit regulates epithelial tight junctions via MRCKα. JCI Insight 6(4):e134881

Basky G et al (2022) XE, XD & XF: what to know about the omicron hybrid variants. CMAJ 194: E654–e655

Basu R et al (2017) Microtubule-assisted altered trafficking of astrocytic gap junction protein connexin 43 is associated with depletion of connexin 47 during mouse hepatitis virus infection. J Biol Chem 292:14747–14763

Bedi S et al (2019) Friend or foe: the role of the cytoskeleton in influenza a virus assembly. Viruses 11(1):46

Bhattacharya B et al (2007) Interaction between bluetongue virus outer capsid protein VP2 and vimentin is necessary for virus egress. Virol J 4:7

Bilinska K et al (2020) Expression of the SARS-CoV-2 entry proteins, ACE2 and TMPRSS2, in cells of the olfactory epithelium: identification of cell types and trends with age. ACS Chem Neurosci 11:1555–1562

Billadeau DD et al (2007) Regulation of T-cell activation by the cytoskeleton. Nat Rev Immunol 7: 131–143

Biswas K et al (2014) Effect of microtubule disruption on neuronal spread and replication of demyelinating and nondemyelinating strains of mouse hepatitis virus in vitro. J Virol 88:3043–3047

Bouhaddou M et al (2020) The global phosphorylation landscape of SARS-CoV-2 infection. Cell 182:685–712.e19

Brian DA et al (2005) Coronavirus genome structure and replication. Curr Top Microbiol Immunol 287:1–30

Buqaileh R et al (2021) Can cilia provide an entry gateway for SARS-CoV-2 to human ciliated cells? Physiol Genomics 53:249–258

Burkard C et al (2014) Coronavirus cell entry occurs through the endo−/lysosomal pathway in a proteolysis-dependent manner. PLoS Pathog 10:e1004502

Caldas LA et al (2020) Ultrastructural analysis of SARS-CoV-2 interactions with the host cell via high resolution scanning electron microscopy. Sci Rep 10:16099

Cao Z et al (2011) Proteomic analysis of chicken embryonic trachea and kidney tissues after infection in ovo by avian infectious bronchitis coronavirus. Proteome Sci 9:11

Cao Z et al (2012) Proteomics analysis of differentially expressed proteins in chicken trachea and kidney after infection with the highly virulent and attenuated coronavirus infectious bronchitis virus in vivo. Proteome Sci 10:24

Carod-Artal FJ (2020) Neurological complications of coronavirus and COVID-19. Rev Neurol 70: 311–322

Chae C et al (2000) Prevalence of porcine epidemic diarrhoea virus and transmissible gastroenteritis virus infection in Korean pigs. Vet Rec 147:606–608

Chen YM et al (2021) Epithelial-mesenchymal transition of absorptive enterocytes and depletion of Peyer's patch M cells after PEDV infection. Virology 552:43–51

Chen H et al (2022) Prediction of coronavirus 3C-like protease cleavage sites using machine-learning algorithms. Virol Sin 37:437–444

Chilvers MA et al (2001) The effects of coronavirus on human nasal ciliated respiratory epithelium. Eur Respir J 18:965–970

Choi S et al (2019) Functional characterization and proteomic analysis of porcine Deltacoronavirus accessory protein NS7. J Microbiol Biotechnol 29:1817–1829

Cortese M et al (2020) Integrative imaging reveals SARS-CoV-2-induced reshaping of subcellular morphologies. Cell Host Microbe 28:853–866.e5
Cui J et al (2019) Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 17:181–192
Del Valle DM et al (2020) An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med 26:1636–1643
den Boon JA et al (2010) Organelle-like membrane compartmentalization of positive-strand RNA virus replication factories. Annu Rev Microbiol 64:241–256
Denes CE et al (2018) Cytoskeletons in the closet-subversion in Alphaherpesvirus infections. Viruses 10(2):79
Dent SD et al (2015) The proteome of the infectious bronchitis virus beau-R virion. J Gen Virol 96: 3499–3506
DeOre BJ et al (2021) SARS-CoV-2 spike protein disrupts blood-brain barrier integrity via RhoA activation. J Neuroimmune Pharmacol 16:722–728
Dewerchin HL et al (2014) Myosins 1 and 6, myosin light chain kinase, actin and microtubules cooperate during antibody-mediated internalisation and trafficking of membrane-expressed viral antigens in feline infectious peritonitis virus infected monocytes. Vet Res 45:17
Döhner K, Sodeik B (2005) The role of the cytoskeleton during viral infection. Curr Top Microbiol Immunol 285:67–108
Döhner K et al (2005) Viral stop-and-go along microtubules: taking a ride with dynein and kinesins. Trends Microbiol 13:320–327
Eisenhut M et al (2020) Pathways in the pathophysiology of coronavirus 19 lung disease accessible to prevention and treatment. Front Physiol 11:872
Elshafei MN et al (2020) The efficacy of colchicine in the management of coronavirus disease 2019: a protocol for systematic review and meta-analysis. Medicine (Baltimore) 99:e21911
Emmott E et al (2010) Quantitative proteomics using stable isotope labeling with amino acids in cell culture reveals changes in the cytoplasmic, nuclear, and nucleolar proteomes in Vero cells infected with the coronavirus infectious bronchitis virus. Mol Cell Proteomics 9:1920–1936
Ezratty EJ et al (2005) Microtubule-induced focal adhesion disassembly is mediated by dynamin and focal adhesion kinase. Nat Cell Biol 7:581–590
Fehr AR et al (2015) Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol 1282:1–23
Ferreira A et al (2020) COVID-19 and herpes zoster co-infection presenting with trigeminal neuropathy. Eur J Neurol 27:1748–1750
Foo KY et al (2015) Interaction between Flavivirus and cytoskeleton during virus replication. Biomed Res Int 2015:427814
Gadanec LK et al (2021) Can SARS-CoV-2 virus use multiple receptors to enter host cells? Int J Mol Sci 22(3):992
Hagemeijer MC et al (2010) Dynamics of coronavirus replication-transcription complexes. J Virol 84:2134–2149
Hao S et al (2020) Long-term modeling of SARS-CoV-2 infection of in vitro cultured polarized human airway epithelium. mBio 11(6):e02852-20
Hatmal MM et al (2020) Comprehensive structural and molecular comparison of spike proteins of SARS-CoV-2, SARS-CoV and MERS-CoV, and their interactions with ACE2. Cell 9(12):2638
Haverkamp AK et al (2018) Experimental infection of dromedaries with Middle East respiratory syndrome-coronavirus is accompanied by massive ciliary loss and depletion of the cell surface receptor dipeptidyl peptidase 4. Sci Rep 8:9778
Helfand BT et al (2011) Vimentin organization modulates the formation of lamellipodia. Mol Biol Cell 22:1274–1289
Hirokawa N (1998) Kinesin and dynein superfamily proteins and the mechanism of organelle transport. Science 279:519–526
Hou W et al (2019) Real-time analysis of quantum dot labeled single porcine epidemic diarrhea virus moving along the microtubules using single particle tracking. Sci Rep 9:1307

Hou YJ et al (2020) SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. Cell 182:429–446.e14
Hou W et al (2021) Dynamic dissection of dynein and Kinesin-1 cooperatively mediated intercellular transport of porcine epidemic diarrhea coronavirus along microtubule using single virus tracking. Virulence 12:615–629
Hu W et al (2016) The epidermal growth factor receptor regulates cofilin activity and promotes transmissible gastroenteritis virus entry into intestinal epithelial cells. Oncotarget 7:12206–12221
Huang C et al (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395:497–506
Iadecola C et al (2020) Effects of COVID-19 on the nervous system. Cell 183:16–27.e1
Jamal M et al (2021) Immune dysregulation and system pathology in COVID-19. Virulence 12: 918–936
Jhan MK et al (2017) Dengue virus infection increases microglial cell migration. Sci Rep 7:91
Jiang F et al (2020) Review of the clinical characteristics of coronavirus disease 2019 (COVID-19). J Gen Intern Med 35:1545–1549
Kalicharran K et al (1995) Involvement of microtubules and the microtubule-associated protein tau in trafficking of JHM virus and components within neurons. Adv Exp Med Biol 380:57–61
Klein S et al (2020) SARS-CoV-2 structure and replication characterized by in situ cryo-electron tomography. Nat Commun 11:5885
Kloc M et al (2020) The multiple sclerosis (MS) drugs as a potential treatment of ARDS in COVID-19 patients. Mult Scler Relat Disord 45:102437
Klok FA et al (2020) Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res 191:145–147
Kong Q et al (2010) Proteomic analysis of purified coronavirus infectious bronchitis virus particles. Proteome Sci 8:29
Lalioti V et al (2022) Cell surface detection of vimentin, ACE2 and SARS-CoV-2 spike proteins reveals selective colocalization at primary cilia. Sci Rep 12:7063
Lee S et al (2015) Functional characterization and proteomic analysis of the nucleocapsid protein of porcine deltacoronavirus. Virus Res 208:136–145
Li SW et al (2012) Correlation between TGF-β1 expression and proteomic profiling induced by severe acute respiratory syndrome coronavirus papain-like protease. Proteomics 12:3193–3205
Li Z et al (2017) Porcine Hemagglutinating encephalomyelitis virus enters neuro-2a cells via Clathrin-mediated endocytosis in a Rab5-, cholesterol-, and pH-dependent manner. J Virol 91(23):e01083-17
Li W et al (2020a) COVID-19, cilia, and smell. FEBS J 287:3672–3676
Li Z et al (2020b) Vimentin as a target for the treatment of COVID-19. BMJ Open Respir Res 7(1): e000623
Li Z et al (2021) A vimentin-targeting Oral compound with host-directed antiviral and anti-inflammatory actions addresses multiple features of COVID-19 and related diseases. MBio 12:e0254221
Lin J et al (2019) Microarray analysis of infectious bronchitis virus infection of chicken primary dendritic cells. BMC Genomics 20:557
Lv X et al (2019) Porcine Hemagglutinating encephalomyelitis virus activation of the integrin α5β1-FAK-Cofilin pathway causes cytoskeletal rearrangement to promote its invasion of N2a cells. J Virol 93(5):e01736-18
Malik YA (2020) Properties of coronavirus and SARS-CoV-2. Malays J Pathol 42:3–11
Mao L et al (2020) Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol 77:683–690
Matschke J et al (2020) Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. Lancet Neurol 19:919–929
May RC et al (2001) Phagocytosis and the actin cytoskeleton. J Cell Sci 114:1061–1077

Meinhardt J et al (2021) Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. Nat Neurosci 24:168–175
Milewska A et al (2018) Entry of human coronavirus NL63 into the cell. J Virol 92(3):e01933-17
Millet JK et al (2012) Ezrin interacts with the SARS coronavirus spike protein and restrains infection at the entry stage. PLoS One 7:e49566
Miranda-Saksena M et al (2018) Infection and transport of herpes simplex virus type 1 in neurons: role of the cytoskeleton. Viruses 10(2):92
Mitchell JA et al (2013) Tropism and pathological findings associated with canine respiratory coronavirus (CRCoV). Vet Microbiol 162:582–594
Naghavi MH et al (2017) Microtubule regulation and function during virus infection. J Virol 91(16):e00538-17
Ng ML et al (2004) Topographic changes in SARS coronavirus-infected cells at late stages of infection. Emerg Infect Dis 10:1907–1914
Nicholls JM et al (2003) Lung pathology of fatal severe acute respiratory syndrome. Lancet 361: 1773–1778
Norris V et al (2021) Role of multifunctional cytoskeletal filaments in Coronaviridae infections: therapeutic opportunities for COVID-19 in a nutshell. Cell 10(17):1818
Owczarek K et al (2018) Early events during human coronavirus OC43 entry to the cell. Sci Rep 8: 7124
Pan P et al (2021) SARS-CoV-2 N protein promotes NLRP3 inflammasome activation to induce hyperinflammation. Nat Commun 12:4664
Pasick JM et al (1994) Distribution and trafficking of JHM coronavirus structural proteins and virions in primary neurons and the OBL-21 neuronal cell line. J Virol 68:2915–2928
Paul D et al (2013) Architecture and biogenesis of plus-strand RNA virus replication factories. World J Virol 2:32–48
Pizzorno A et al (2020) Characterization and treatment of SARS-CoV-2 in nasal and bronchial human airway epithelia. Cell Rep Med 1:100059
Priestnall SL et al (2009) Quantification of mRNA encoding cytokines and chemokines and assessment of ciliary function in canine tracheal epithelium during infection with canine respiratory coronavirus (CRCoV). Vet Immunol Immunopathol 127:38–46
Ramos I et al (2020) Vimentin as a multifaceted player and potential therapeutic target in viral infections. Int J Mol Sci 21(13):4675
Ren JL et al (2020) Traditional Chinese medicine for COVID-19 treatment. Pharmacol Res 155: 104743
Ren X et al (2021) COVID-19 immune features revealed by a large-scale single-cell transcriptome atlas. Cell 184:1895–1913.e19
Rho SS et al (2017) Dynamic regulation of vascular permeability by vascular endothelial cadherin-mediated endothelial cell-cell junctions. J Nippon Med Sch 84:148–159
Ross JL et al (2008) Cargo transport: molecular motors navigate a complex cytoskeleton. Curr Opin Cell Biol 20:41–47
Rüdiger AT et al (2016) Tubulins interact with porcine and human S proteins of the genus Alphacoronavirus and support successful assembly and release of infectious viral particles. Virology 497:185–197
Schurink B et al (2020) Viral presence and immunopathology in patients with lethal COVID-19: a prospective autopsy cohort study. Lancet Microbe 1:e290–e299
Scott BM et al (2022) Predicted coronavirus Nsp5 protease cleavage sites in the human proteome. BMC Genom Data 23:25
Shi X et al (2020) Unidirectional regulation of vimentin intermediate filaments to Caveolin-1. Int J Mol Sci 21(20):7436
Snijder EJ et al (2003) Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J Mol Biol 331:991–1004
Snijder EJ et al (2006) Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. J Virol 80:5927–5940

Snijder EJ et al (2020) A unifying structural and functional model of the coronavirus replication organelle: tracking down RNA synthesis. PLoS Biol 18:e3000715
Sodeik B et al (1997) Microtubule-mediated transport of incoming herpes simplex virus 1 capsids to the nucleus. J Cell Biol 136:1007–1021
Suikkanen S et al (2003) Exploitation of microtubule cytoskeleton and dynein during parvoviral traffic toward the nucleus. J Virol 77:10270–10279
Sun M et al (2017) Identification of two mutation sites in spike and envelope proteins mediating optimal cellular infection of porcine epidemic diarrhea virus from different pathways. Vet Res 48:44
Suomalainen M et al (1999) Microtubule-dependent plus- and minus end-directed motilities are competing processes for nuclear targeting of adenovirus. J Cell Biol 144:657–672
Suprewicz Ł et al. (2021) Extracellular vimentin as a target against SARS-CoV-2 host cell invasion. bioRxiv
Suprewicz Ł et al (2022) Extracellular vimentin as a target against SARS-CoV-2 host cell invasion. Small 18:e2105640
Surjit M et al (2004) The SARS coronavirus nucleocapsid protein induces actin reorganization and apoptosis in COS-1 cells in the absence of growth factors. Biochem J 383:13–18
Thakur KT et al (2021) COVID-19 neuropathology at Columbia University Irving medical center/New York Presbyterian hospital. Brain 144:2696–2708
Tilley AE et al (2015) Cilia dysfunction in lung disease. Annu Rev Physiol 77:379–406
Ukmar-Godec T et al (2020) Biomolecular condensation of the microtubule-associated protein tau. Semin Cell Dev Biol 99:202–214
Van Hamme E et al (2008) Clathrin- and caveolae-independent entry of feline infectious peritonitis virus in monocytes depends on dynamin. J Gen Virol 89:2147–2156
Villarreal LY et al (2007) Orchitis in roosters with reduced fertility associated with avian infectious bronchitis virus and avian metapneumovirus infections. Avian Dis 51:900–904
Wang J et al (2009) Interaction of the coronavirus infectious bronchitis virus membrane protein with beta-actin and its implication in virion assembly and budding. PLoS One 4:e4908
Wang IH et al (2018) Imaging, tracking and computational analyses of virus entry and egress with the cytoskeleton. Viruses 10(4):166
Wang H et al (2019) Infectious bronchitis virus entry mainly depends on clathrin mediated endocytosis and requires classical endosomal/lysosomal system. Virology 528:118–136
Wang F et al (2020a) Long-term respiratory and neurological sequelae of COVID-19. Med Sci Monit 26:e928996
Wang J et al (2020b) Dynamics of transmissible gastroenteritis virus internalization unraveled by single-virus tracking in live cells. FASEB J 34:4653–4669
Wang JY et al (2021) An autoantigen profile of human A549 lung cells reveals viral and host etiologic molecular attributes of autoimmunity in COVID-19. J Autoimmun 120:102644
Welte MA (2004) Bidirectional transport along microtubules. Curr Biol 14:R525–R537
Wen Z et al (2020) Cytoskeleton-a crucial key in host cell for coronavirus infection. J Mol Cell Biol 12:968–979
Xu K et al (2013) Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. Science 339:452–456
Xu Z et al (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 8:420–422
Yamamoto K et al (2021) Rap1 small GTPase regulates vascular endothelial-cadherin-mediated endothelial cell-cell junctions and vascular permeability. Biol Pharm Bull 44:1371–1379
Yu YT et al (2016) Surface vimentin is critical for the cell entry of SARS-CoV. J Biomed Sci 23:14
Zandi F et al (2021) Rabies virus matrix protein targets host actin cytoskeleton: a protein-protein interaction analysis. Pathog Dis 79(1):ftaa075
Zhang X et al (2013) Identification of cellular proteome using two-dimensional difference gel electrophoresis in ST cells infected with transmissible gastroenteritis coronavirus. Proteome Sci 11:31

Zhang X et al (2015) Identification of the interaction between vimentin and nucleocapsid protein of transmissible gastroenteritis virus. Virus Res 200:56–63
Zhang Y et al (2019) The role of host cytoskeleton in Flavivirus infection. Virol Sin 34:30–41
Zhang Q et al (2020) Heparan sulfate assists SARS-CoV-2 in cell entry and can be targeted by approved drugs in vitro. Cell Discov 6(1):1–14
Zhao S et al (2014) Transmissible gastroenteritis virus and porcine epidemic diarrhoea virus infection induces dramatic changes in the tight junctions and microfilaments of polarized IPEC-J2 cells. Virus Res 192:34–45
Zhou F et al (2020) Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395:1054–1062
Zhu N et al (2020) Morphogenesis and cytopathic effect of SARS-CoV-2 infection in human airway epithelial cells. Nat Commun 11:3910
Zubair AS et al (2020) Neuropathogenesis and neurologic manifestations of the coronaviruses in the age of coronavirus disease 2019: a review. JAMA Neurol 77:1018–1027