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Review

Activation of Host Cellular Signaling and Mechanism of EV71 Viral Proteins Associated with Hand, Foot and Mouth Disease

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Abstract: Enteroviruses are members of *Pichornaviridae* family consisting of human enterovirus group A, B, C, and D as well as nonhuman enteroviruses. Human enterovirus type 71 (EV71) has emerged as a major cause of viral encephalitis Hand, foot, and mouth disease (HFMD) in children worldwide especially in the Asia-Pacific region. EV71 and coxsackievirus A16 are two viruses responsible for HFMD which are members of group A enterovirus. The identified EV71 receptors provide useful information for understanding viral replication and tissue tropism. Host factors interact with the internal ribosome entry site (IRES) of EV71 to regulate viral translation. However, the specific molecular features of the EV71 genome that determine virulence remain unclear. Although an EV71 vaccine has been currently approved, there is no effective therapy for treating EV71 infected patients. Therefore, understanding the host-pathogen interaction could provide the knowledge in viral pathogenesis and further benefit in the anti-viral therapy development. The aim of this study was to investigate the latest findings about the interaction of viral ligands to the host receptor as well as the activation of immune related signalling pathways for the activation of innate immunity and involvement of different cytokines and chemokines in the host pathogen interaction of EV71 along with interaction of viral proteins, mainly 2A and 3C protease, and Interferons production/signaling pathway and their inhibitory effects.

Keywords: enterovirus; viral proteins; signalling pathways; host-pathogen interaction

1. Introduction

Enterovirus71 (EV71) is a sense, single stranded, non-enveloped RNA virus belonging to *Enterovirus* genus of family *Picornaviridae* which is the main causative agent of Hand, Foot and Mouth disease (HFMD) related to serious health complications such as neurological and cardiovascular complexities that commonly affects infants and children [1]. This disease is manifested by a wide range of symptoms such as fever, rash, diminished appetite, and mouth ulcers followed by opportunistic critical clinical conditions, such as neurological dysfunction, cardio-respiratory collapse, or worse mortality [2]. The 7.4kb long genome of EV71 is a single positive strand RNA has an open reading frame (ORF) that codes for a polypeptide of 2194 amino acids followed by the 3' non-translated region with poly-adenylated (poly-A) tail. In the case of the EV71 genome, an internal ribosomal entry site (IRES) has been observed with the facility of cap-independent translation in the viral protein. The ORF has a single wide polyprotein having around 2100 amino acids and separated into three regions (P1-P3). A variety of processing activities in the polyprotein leads to the cleavage of polyprotein and viral non-structural and structural proteins. The P1 region constitutes the capsid virus encodes the four structural proteins such as VP1, VP2, VP3, and VP4 (VP1-VP4). The virus replication's most direct results are non-structural P2 proteins (3Dpol, 3CDpro, 3Cpro, 3A, 3AB, and 3B) [3]. The capsid of the EV71 is developed through the protomeric components of 60 units, involved with the four structural protein polypeptides VP1-VP4. These are encoded in the genome P1 area. P3 and P2 encodes seven nonstructural proteins (3A-3D and 2A-2C), followed by

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poly-A residues at 3'UTR. Among these, it has been demonstrated that the viral protease 3C is implicated in numerous pathogenic process of EV71. The connection site Gln-Gly of P2-P3 can be cleaved by EV71 3C proteases [4].

Host-pathogen interaction has provided the scope for the development of host receptors based on the identification of specific routes for the interaction. It is important to understand that the host-viral relationship has been responsible for promoting the viral protein to facilitate the condition of generating a host factor to interact with the viral protein. The process of infection has been associated with the condition of the host and its immune response [5]. When an EV71 infection occurs, inflammation is known to play a critical role. This is always characterized by an infiltration of inflammatory cells, a release of pro-inflammatory cytokines and chemokines, edema, and vascular leakage [6]. It has been widely appreciated that EV71 infection can result in complex inflammation on the sites accompanied by immune evasion, multiple immune cell responses, and proinflammatory cytokine release [7,8]. It has been reported that many cellular signaling pathways are involved in EV71 replication and inflammatory pathogenesis [9-11]. In this review, we mainly focus on the major cellular signaling pathways involved in EV71induced antiviral innate immunity and inflammatory responses.

2. Molecular Mechanism Associated with Host-Pathogen Interaction in Ev71

Molecular mechanism involved with the host-pathogen receptor has been associated with the identification of the cell surface receptor development for entrapment of the virus. It is important to understand that five different molecules in the cell surface of the host, which provides the opportunity to support the aspect of the host-pathogen interaction by the involvement of possible cell surface receptor. The five individual molecules for cell surface receptors are as follows, Scavenger receptor B2 (SCARB2), heparan sulfate, Sialylated glycan, P-selectin glycoprotein ligand-1 (PSGL-1), and annexin II [12]. The SCARB2 protein has provided the cell-surface receptor for the development of binding sites, which provides the evidence for the critical receptor development for the involvement of host-pathogen interactions. Moreover, EV71 has been involved in the endocytic pathway for the progress of infection (Figure 1).

The viral capsid is an important part of the molecular mechanism for host-pathogen interaction to promote the development of the natural lipids that helps to uncoat the viral particle to release the genome inside the host. Mechanism of action of the viral capsid is developed through the conformational changes in the capsid followed by the removal of lipids that have provided an opportunity to release VP1 and VP4 N-termini to support the aspect of uncoating viral genome. However, in the case of human EV71 and Coxsackievirus A16, two types of membrane proteins such as SCARB2 and PSGL-1have provided the condition for the development of the receptors. The important step of uncoating the viral genome has been developed in the area of endosome, followed by the acidification to support the aspect of host-related interaction. However, it is important to understand that the acidic environment such as pH=4 has been associated with the development of scope for EV71 related host-pathogen interaction by promoting the releasing of the viral genome [13].

The mechanism of the molecular context of the host-pathogen interaction has provided the conceptual idea regarding the viral replication inside the host cell. The immune response of the host can be determined through the evaluation of the replication. According to the development of the scope for viral protein development, the host translation system translates the viral genome inside the host cytoplasm by the action of FUBP1 followed by an internal ribosome entry-transacting factor [14]. The internal changes that occurred due to the outbreak of the viral protein have provided the condition for suppressing antiviral and cap-dependent transcription by the presence of different signalling components such as PI4KB and immune responses, RIG-I as well as MAVS. This interactions have been developed through the involvement of SiRNA libraries of endocytosis, serine or threonine kinase as well as the genes of membrane trafficking.

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On the other hand, it is required to understand that a post-transcriptional gene expression regulation has been associated with the development of MicroRNA, which identifies the host and viral particle complexity during attachment. On the other hand, few genetic expressions have played an inhibitory role during viral replication. A post-transcriptional factor named as miR-23b has provided the opportunity for the down-regulated interactions. Meanwhile, 3'UTR of EV71 is a conserved sequence that has been regulated through the miR-23b. Moreover, the effectiveness of the virus replication within the host cell has been associated with the up-regulation of the hsa-miR-494-3p level, which regulates the PI3K/Akt signaling pathway [15]. Furthermore, hsa-miR-141 expression targets translation initiation factor eIF4E based on the cap-dependent and cap-independent translation within the host system [16]. In this case, host miRNA has been associated with the potential interaction development to inhibit the action for viral propagation. Moreover, gene sequence hsa-miR-548 has been responsible for the development of host antiviral responses by targeting the IFN-λ1 factor within the host organism.

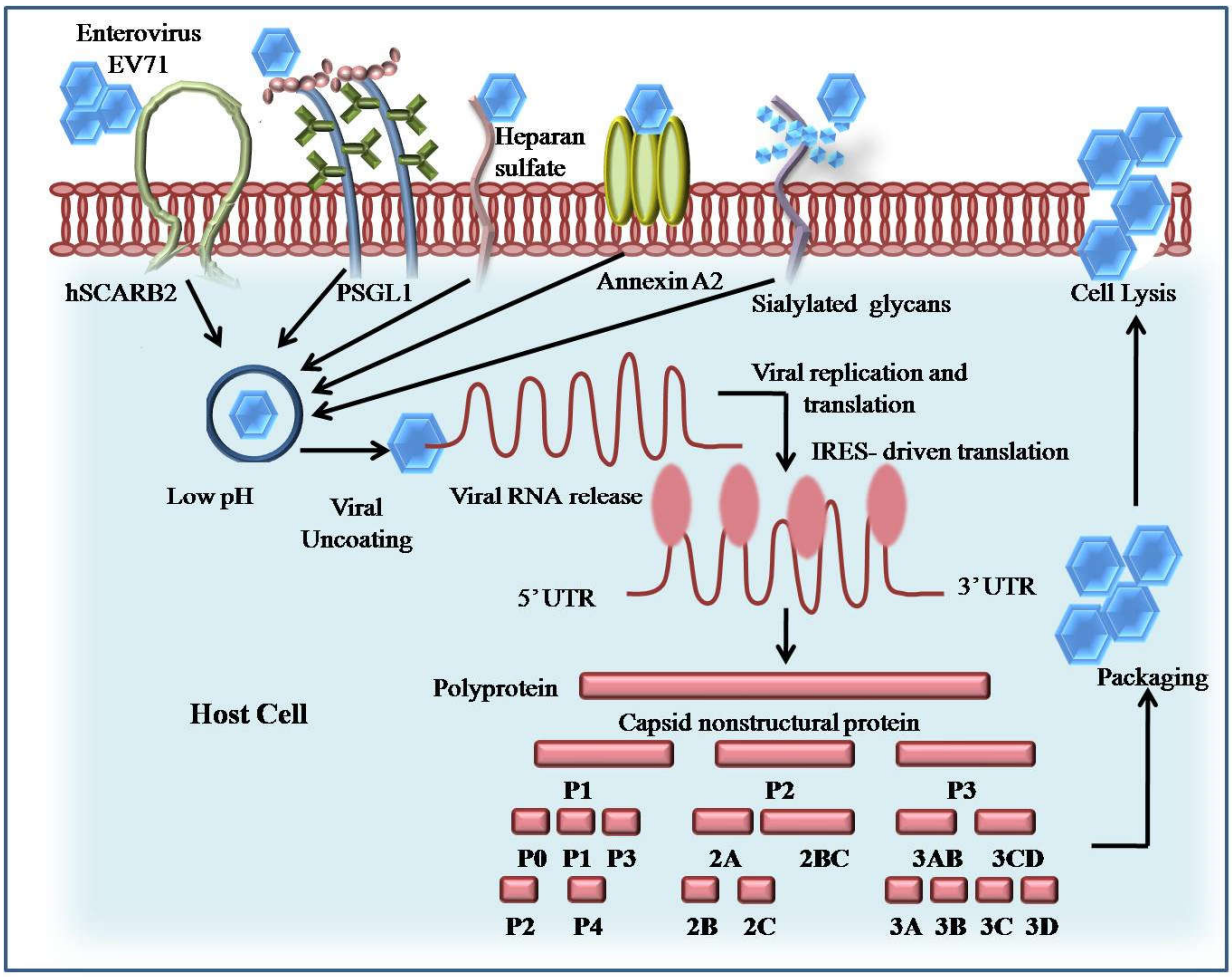

Figure 1. Invasion and replication of EV71 inside the host cell. EV71 enters into the host cell by binding with specific receptors and release the viral RNA inside the cell. The mechanism of immunogenic evasion by the EV71 inside the host by escaping from innate immunity through the inappropriate recognition of the Cytotoxic T lymphocytes based on the conformational change in the antigen structure followed by viral antigen epitope. A single polyprotein is produced by IRES-driven translation which is then cleaved into proteins by proteolysis.

3. Immune Cells Involvement during Evasion Process

Host-pathogen interaction has been associated with the development of the activation of immunogenic responses inside the body of the host organism. However, invasion of the foreign particle or pathogen inside the host organism activates the conflicts between

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the innate immune system and adaptive immune system [17]. However, it is important to understand that the immunogenic defence mechanism of the host organism has been involved with the development of the condition for activating the adaptive immune system throughout the functional condition of the innate immunity [18]. Innate immunity is responsible for developing the scope of the physiological responses to facilitate the activation of the pathogenic evasion inside the host organism through various immunogenic cells such as macrophages, neutrophils, dendritic cells, and natural killer cells. Moreover, it can be observed that the innate immune system of the pathogen provides scope for the identification of Pathogen-associated molecular patterns (PAMPs) through the development of pathogen-recognition receptor (PRR) on the cellular organelle and membrane to identify the invasion of the foreign particle.

3.1. Apoptosis

As soon as EV71 enters the host cells, cap-independent translation of the viral RNA starts. The recruitment of the generated viral proteins triggers the replication of viral RNA. Apoptosis usually starts as the viral lineage is being replicated by the host or viral components [19]. According to recent studies, autophagy, particularly in the case of neuronal cells, may also be responsible for the cell death associated with the EV71 infection. Increasing evidence indicates that EV71 infection induces apoptosis in a range of cell lines, including HeLa , rhabdomyosarcoma (RD) , Jurkat , SK-N-MC , glioblastoma SF268 ce, Vero , and the human microvascular endothelial cells [20-23]. Numerous investigations have demonstrated that the activation of caspases by the proteolytic activity of EV71 3C and 2A can result in apoptosis. EV71 also induces apoptosis in a variety of host cells via many apoptotic mechanisms. Caspase 8 activation and Bid cleavage produced by EV71 infection in non-neural cells, whereas the mitochondrial route causes apoptosis in neural cells [21]. In addition, Chen et al., 2006 found that EV71infected brain cells activated Cdk5. The increased FasL expression that has been observed in Jurkat T cells infected with EV71 may help to explain the decrease in T cells [23]. Notably, it has been demonstrated that very early in the course of infection, EV71 infection activates the PI3K/AKT and MAPK/ERK signalling pathways. The stimulation of these pathways inhibits GSK3b activity and may delay host apoptosis [24].

Infection with EV71 has been found to change cellular signaling cascades such as MEK/ERK and PI3K/Akt in order to modify the cellular functioning and the virus life cycle [24-26]. The PI3K/Akt pathway activation may be implicated in the regulation of viral protein production and host cell apoptosis [25]. Activated ASK1 is a substrate for Akt phosphorylation, which is related with a reduction in stimulated ASK1 kinase activity. Akt inhibits ASK1 activity, resulting in the observed restricted JNK phosphorylation. EV71 infection of RD cells activated the pro-apoptotic protein Bax, as shown by a conformational shift and translocation from the cytosol to mitochondria, which coincided with the release of Cytochrome C. However, the PI3K/Akt survival pathway in RD cells restricted the degree of EV-71 induced JNK activation and JNK- mediated death via Ask1 phosphorylation [27] (Figure 2).

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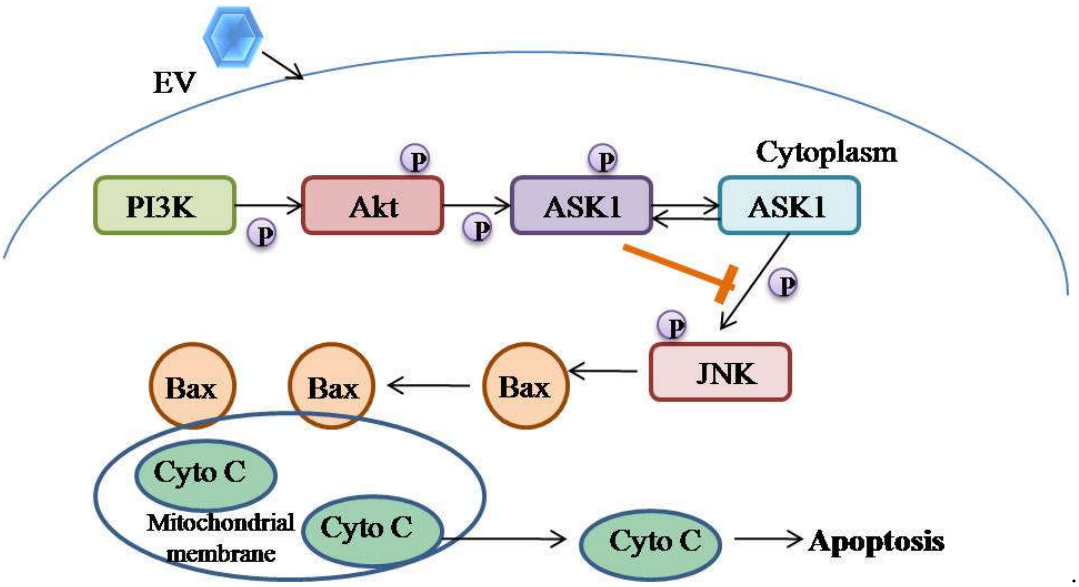

Figure 2. EV71 induced JNK- mediated apoptosis pathway inhibited by PI3K/Akt activation through phosphorylating and negatively regulating ASK1.

3.2. Autophagy

In the host defense against a variety of intracellular pathogens such as bacteria, parasites and viruses, autophagy probably plays a significant role. Double-membrane autophagosomes are seen in RD and neuroblastoma (SK-N-SH) cells that have been infected with the respective virus. The expression of the autophagosomal marker LC3 II and the viral proteins are positively correlated [19]. The mechanisms by which EV71 induces autophagic processes should be understood in order to design effective treatment plans for EV71related disorders. Toll-like receptor (TLR) activation or induced endoplasmic reticulum (ER) stress can both cause autophagy [28]. Jheng et al. showed that EV71 infection causes eIF2a to become phosphorylated as well as the over expression of the ER-resident chaperone proteins BiP and calreticulin [29]. Since inactivated EV71 is devoid of these skills, ER stress might be brought on by viral replication rather than viral entrance or attachment that leads to development of autophagosomes.

3.3. Innate immunity

The activation of PRR pathway, which results in the generation of IFNs, is typically linked to host innate immune response. TLRs and DExD/H box helicases like RIG-I and MDA5 are among the PRRs [30]. Many cells express TLR, notably TLR3, 7, and 8 are capable of detecting RNA inside cells. Therefore, these TLRs are crucial for detecting viral incursions. MDA5 and RIG-I are homogenous IFN-inducible proteins that are triggered by RNA species detection. IFNs that are created that have antiviral properties because they can attach to receptors on the small cell or on nearby cells. According to a study, EV71 3C can connect with RIG-I. This interferes with IPS-I recruitment, which in turn prevents IRF3 from being activated [31].

3.4. Acquired Immunity

The immune system is triggered by viral infection to eliminate the invasive pathogens. Age is a crucial risk factor for this disease. Children under the age of five with undeveloped immune systems are particularly vulnerable to EV71 infection [32, 33]. Age-related increases in EV71 antibody titters have been demonstrated by retrospective serological research [34]. Thus, this might provide an explanation for why young toddlers are vulnerable. Eighty percent of individuals with hand, foot, and mouth disease whose antibody titters were examined tested positive for the antiEV71 antibodies within a day of developing their illness [35]. But there is no connection between the severity of the illness

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and the antibody response [35, 36]. Therefore, the fate of an EV71 infection may be significantly influenced by the cellular immune responses.

3.5. Immune evasion

The process of immunogenic evasion has been responsible for developing the scope for increasing the probability of the spreading of pathogens by escaping the immunogenic responses within the host organism. Host immune pressures are responsible for formulating the condition for the development of immunological evasion caused by EV-71 to support the spreading of the virus inside the host cell.

4. Characterization of Coding and Non-Coding Region of Ev71

4.1. VP1

VP1 is a primary 297- amino acid capsid protein present at the most exterior portion. During EV71 infection, VP1 binds to immunoglobulin–like receptors PSGL-1 and SCARB2. Receptor biding requires PSGL-1 binding by regulating VP1-244K exposure. When EV71 virions attach to the receptors, they undergo a two-step uncoating process, first creating an enlarged, altered "A-particle" that expels VP4 and exposes the N-terminus of VP1. Second, the A-particle capsid develops a breach in the endosomal membrane and opens a two-fold channel near the icosahedral axis to facilitate genome release. The crystal structure of the EV71 uncoating intermediate also revealed that the VP1 N-terminal extensions (1-71residues) interact with the viral RNA. Furthermoe, VP1 activates ER stress and autophagy may enhance the over expression of cell surface-exposed calreticulin (Ecto-CRT), a key mediator of primary phagocytosis. Heparan sulfate is used as an attachment receptor by EV71, and neither VP1-98 nor VP1-145 can modify heparan binding. Galectin-1, a soluble beta-galactoside binding lectin, may interact with 1 VP1 via carbohydrate residues before being released and attached to another cell surface alongside the virus. Vimentin on the cell surface is an attachment receptor that binds to VP1 via N-terminus to enhance the infection. The VP1 protein activates calmodulin dependent protein kinase II, which phosphorylates the N-terminal region of vimentin on serine 82 during infection. Vimentin phosphorylation and rearrangement may improve RV-71 replication by playing structural roles in replication factor production.

4.2. VP2-VP3

EV71 virus capsid proteins VP2 and VP3, which are important parts of the shell protein, are associated with the antigenicity of the virus. Thus, VP2 and VP3 may be potential candidates with structures similar to that of VP1, and VP2 (amino acids 142–146) contains a single, linear, non-neutralizing epitope, which is located in the E–F loop of the VP2 protein [37, 38]. The structure of VP2, VP3 and VP1 is the same; they form an eight stranded anti-parallel b-barrel structure in the shape of a wedge that favors packing. The shape resembles b-sandwich "jelly roll" fold. The linking loops and C-termini on the outer surface of the capsid are the key structural changes. The "puff" is the most noticeable surface loop in VP2 and the "knob" is the greatest protrusion on the surface in VP3. VP3 consists of 245 amino acids, among which the amino acids 59–67 of VP3 are more highly conserved between the subgenogroups, compared to VP1. There is a conserved conformational epitope on the "Knob" region of VP3, which makes it an ideal target for a diagnostic or therapeutic mAb. 5H7, a therapeutic IgG antibody, was recently demonstrated to target a conformational epitope mapped to highly conserved amino acid position 74 of VP3.

4.3. VP4

VP4 is made up of 69 amino acids, an extended conformation and is present inside the virion. The VP4 package is embedded in the virus shell, is strongly linked to the virus score, and has an extended spatial conformation characteristic, which act as a bridge between the inside and outside of the virus [37, 39]. The spatial configuration of the virus changes when it connects to the receptors. The viral shell is eventually destroyed, the

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viral genomic RNA is released into the cytoplasm, and the viral translation begins. The viral genomic RNA, known as mRNA, is the starting point for polymeric protein. The VP4 N-terminal myristoylation signal (MGXXXS) is play an important role in EV71 replication. Because the VP4 genome is more conserved that the VP1, VP2 and VP3 genes, studies on EV71 vaccine focused on neutralizing epitopes of VP4 protein [40].

4.4. 2A-2C & 3A-3D

The EV71 2A protease has cysteine protease activity and 150 amino acid residues; an enzyme that cleaves at its own N-terminus at the polyprotein junction between VP1 and 2A. By cleaving the elongation factor elF4G and facilitating EV71 replication, 2Apro suppresses host cap-dependent protein synthesis [41, 42]. By cleaving mitochondrial antiviral protein and RIG-1 like receptor MDA5, the 2A protease can lower IFN-1 receptor protein levels and block interferon regulatory factor 3 (IRF3) signalling, allowing the virus to evade the immune response [43]. 2A inhibits IFN- γ signaling by lowering serine phosphorylation of signal transducer and activator of transcription 1 (STAT 1) [43-45].

The EV71 2B protein, a tiny hydrophobic ion channel protein with 99 amino acid residues, that may mediate a chloride-dependent rather than calcium-dependent channel to regulate viral replication [46]. The C-terminal portion of 2B (63-80 amino acid) is thought to be crucial for mitochondrial localization, and causes cell death via interacting with and recruiting Bax, a proapoptotic protein, as well as triggering Bax conformational activation. A 14-amino acid hydrophilic domain in the N-terminus of 2B is required for Bax binding and subsequent activation (Figure 3) [47].

2C protein having 329 amino acid residues is one of the most highly conserved non-structural proteins. At the C-terminal region, it contains an ATPase domain, a zinc finger structure, and an alpha helix [48]. In vitro, the 2C ATPase, an RNA helicase that unwinds RNA helices in an ATP-dependent manner and an RNA chaperone independent of ATP, that promotes EV71 RNA production. Through PP1-docking motif, the N-terminus of 2C (1-125 amino acid) interacts with all isoforms of the protein phosphatase 1 (PP1) catalytic subunit, which is effective for EV71 2C-mediated suppression and NF-κB activation (Figure 3) [26].

4.5. 2A and 3C Proteases

2A protease is initially translated during the translation of enterovirus polyprotein non-structural region (P2). It then self cleaves to split from the P2 and P1 areas. This is followed by the P3 region, which includes the second protease 3C, which is responsible for 8 out of 10 cleavages of the viral polyprotein [49]. Different enterovirus genotypes showed roughly 50% to 75% sequence similarity in 2A and 3C. The twisted β-barrels stack perpendicular to each other from the tertiary structure of 3Cpro. The domains contribute to the formation of a catalytic site, which includes histidine, aspartic acid and cysteine in 2Apro and histidine, glutamic acid and cysteine in 3Cpro [50]. The P1 location, which is mainly glycine, is a critical for 2A protease. P2 position is highly significant after P1, which is commonly identified by threonine and asparagines. P2 might be proline, alanine, or phenylalanine, and P4, which is generally a location for leucine or threonine. P1 in the substrate sequence show the greatest conservation for 3Cpro [51]. For P1, the present amino acid is glutamine or glutamate, whereas, for P2 it is glycine, asparagines, or serine. 3Cpro has been identified as a promising target for antiviral medicines because the enteroviral polyprotein that contains many cleavages sites unique for protease and plays an important role in virus maturation [52].

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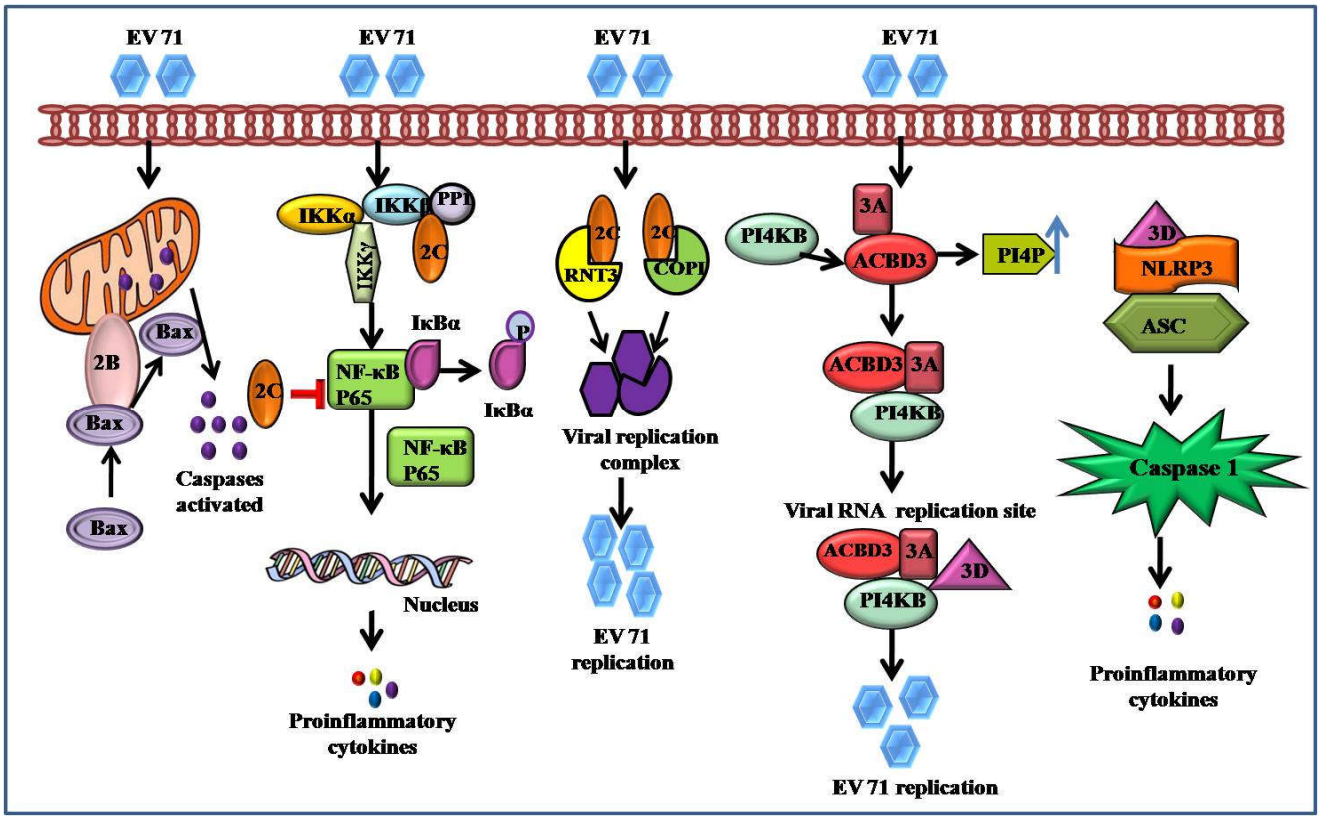

Figure 3. A graphical illustration of the role of each protein in EV71 induced signaling pathways. By directly interacting and activating the pro-apoptotic protein Bax, 2B triggers cell apoptosis. The over expression of ant-apoptotic protein Bcl-XL prevent 2B-induced caspase activation and cytochrome C release. 2C protein exhibits both RNA- and membrane-binding activity, interacts with reticulon 3 (RNT3) then combines with double-stranded RNA viruses to form viral replication complex and participate in viral replication. By interacting with 3A protein, the Golgi protein acylcoenzyme A binding domain- containing 3 (ACBD3) facilitates EV71 replication, and EV71 triggers the generation of IL-1β by activating NLRP3 inflammasome.

5. Immune Related Signaling Pathways Associated with Ev71

5.1. MAPK Signaling Activated by EV71

Mitogen activated protein kinase (MAPK) belongs to a family of Serine/threonine protein kinase, highly conserved throughout eukaryotes and involved in variety of cellular function such as inflammation, stress, cell growth, cell development, motility, differentiation, proliferation and death by phosphorylated transcription factors and enzymes [53]. Mitogen activated protein kinase kinase kinase (MAPKKK), Mitogen activated protein kinase kinase (MAPKK), Mitogen activated protein kinase (MAPK) are all sequentially phosphorylated in order to transmit the MAPK signal. There are six sub-family of MAPK has been identified in mammalian cells to date such as JNK1/2/3, ERK 1/2, p38 MAPK (p38α/β/γ/δ), ERK 7/8,ERK3/4 and ERK5/big MAPK1 (BMK1) [54]. In human cells, EV71 infection induced intrinsic apoptosis and p38-mediated proinflammatory cytokines. Leong et al., 2015 discovered that EV71 infection led to the activation of misshapen/Nck-interacting kinase (NIK)-related kinase (MINK), which in turn increased the phosphorylation of p38. TAK1 is a participant in MAPKKK [55]. To prevent cytokine synthesis brought on by an EV71 infection, 3Cpro can affect the TAK1 complex proteins. As a result, EV71 infection on the one hand causes an inflammatory reaction by activating MAPK pathways [56-57]. On the other side, in order to engage with the inflammatory response, EV71 may block MAPK pathways (TAK1 signalling).

The JNK1/2 and p38 MAPK signaling pathways regulate proinflammatory cytokine release as well as EV71 replication [58, 59]. However, there is lack of evidence with regard to activation of JNK1/2 and p38 MAPK in immature dendritic cells (iDCs) during EV71

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infection. JNK1, JNK2, and JNK3 are the three different genes that code for the mammalian JNKs, and studies have shown that these proteins are highly active in response to cytokines, UV radiation, growth factor deficiency, DNA-damaging chemicals, and viral infection [60, 61]. While JNK3 is exclusively present in the brain and testis; JNK1 and JNK2 are expressed in the majority of cell types [62]. With prolonged infection, EV71 infection boosted the mRNA levels of MEK4, MEK7, and JNK1/2 and improved JNK1/2 phosphorylation [63].

The term "p38 MAPK" refers to four distinct isoforms of the protein [64]. Dual MAP2Ks (such as MEK3 and MEK6, etc.) and a number of MAP3Ks, including MTK1, MLK2/MST, MLK3, ASK1, and TAK1, have been observed to activate p38 MAPK kinases [65, 54].

When EV71 infects iDC, it can activate the JNK1/2 and p38 MAPK signalling pathway cascades, phosphorylate downstream molecules like c-Jun and c-Fos, and promote the release of proinflammatory cytokines. Proinflammatory cytokines like IL-6, TNF, and IFN are frequently brought on by oxidative stress, cytokines, and viral infection. These elements play an important role in inducing host cell damage, chronic inflammation, and other immune responses. In response to EV71 infection, DCs may release a variety of cytokines [66].

5.2. EV71 Induces Phosphatidylinositol 3-Kinase (PI3K) Signaling

Numerous Akt target proteins are phosphorylated as a result of Akt pathway activation, mediating a variety of cellular functions. Apoptosis signal-regulating kinase 1 [67], caspase-9, the transcription factors Forkhead, BAD, and NF-B are among the targets of Akt that have been linked to the regulation of cell survival [68]. The Akt pathway should typically activate a number of its downstream substrates. Inhibition of the PI3K/Akt pathway enhanced JNK phosphorylation and the JNK-mediated apoptosis during infection. Moreover, PI3K/Akt pathway phosphorylated apoptosis signal-regulating kinase 1 (ASK1) and negatively regulated the ASK1 activity. Knockdown of ASK1 significantly decreased JNK phosphorylation, which inhibit the ASK1-mediated JNK activation. Collectively, these data reveal that activation of the PI3K/Akt pathway limits JNK-mediated apoptosis by phosphorylation and inactivating ASK1 during EV71 infection [27].

Though the activation of the downstream protein kinase AKt, PI3K signaling is well established for controlling cellular growth and proliferation and for playing a significant role in activating the inflammatory responses. According to some theories, EV71 stimulates the PI3K/Akt pathway, which in turn controls the transcription of pro-inflammatory cytokines and may release in response to PI3K/Akt activation in human RD cells. Akt is phosphorylated by EV71 in a manner that PI3K dependent. The PI3K/Akt pathway can be activated by EV71 infection, further influencing the inflammatory response.

5.3. EV71 Activates Calcium (Ca2+)-Dependent Signaling

Calpains are a family of calcium (Ca2+) dependent cysteine proteases. Both calpain 1(μ-calpain) and calpain 2 (m-calpain), which are ubiquitous isoforms localized in the cytosol and mitochondrion respectively, are activated *in-vitro* by micromolar and millimolar amounts of Ca2+. Due to the fact that a large number of calpain substrates are connected to pro-apoptotic state, and their activity has been linked to apoptosis [69-71]. Additionally Ca2+ is an important regulator of cell survival; persistent Ca2+ elevation inside of cells may activate calpains after exposure to some anti-cancer drug, leading to apoptotic cell death.

Ca2+ is a ubiquitous second messenger that controls variety of processes in eukaryotic cells. Infected cells with EV71 have been shown to have higher levels of mitochondrial Ca2+.Calpain activation through Ca2+ flow is crucial for triggering an apoptosis inducing factor (AIF), caspase-independent, apoptotic pathway during EV71 infection. In EV71 infected cells, the administration of ruthenium red, a mitochondrial Ca2+ influx inhibitor, dramatically inhibit calpain activation and AIF cleavage. Ca2+ homeostasis requires

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calmodulin-dependent protein kinase II (CaMKII). CaMKII can be activated by the EV71 VP1 protein. If activated, it phosphorylates serine 82 in the N-terminal domain of vimentin, which subsequently functions structurally in viral propagation. By triggering Ca2+ dependent signaling, EV71 infection promotes both virus replication and cell death.

5.4. EV71 Encoded Proteases Inhibit MAVS-Mediated Antiviral Signaling

The innate immune response is triggered as the initial line of defense against viral invasion when virus infects host cells. Host PRR detect PAMPs, which causes the release of type-1 interferon and proinflammatory cytokines that activate host adaptive immunity and generate an antiviral state in host cells. Generally, there are three phases of anti-viral innate immunity; I: the initiation phase, where PRR binds to viral RNA and recruits particular signaling adaptor molecule; II: the signal transduction phase, in which adaptor molecule transmit signaling to activate IKK-related kinase, that facilitate transcription factors like interferon regulatory factor 3 (IRF3) and nuclear factor B (NF-B) and III: the effector phase, where the Type 1 IFN cause the synthesis of interferon stimulated genes by activating the signal transducer and activator of transcription (STAT) pathway. Membrane-bound TLRs) and cytoplasmic sensors, such as retinoic acid induced gene-I (RIG-I) and melanoma differentiation associated gene, are used to detect RNA viruses (MDA-5). RIG-I and MDA-5 both include RNA helicase domains and use the mitochondrial antiviral signaling protein to transduce signals.

5.5. EV71 Infection-Associated IFN Signaling

Interferons are essential for limiting viral replication and dissemination in mammalian responses to viral infections [72]. Based on how they interact with their receptors, IFNs are divided into three types; type I, type II, type III. Upon viral infection, type I IFNs are generated, which then activates a number of antiviral effectors to build a defense network against viral replication [73]. It has been determined that type I IFN subtype IFN-4, IFN-6, IFN-14, IFN-16 suppress the replication of EV71. In particular, IFN-14 prevented virus replication 20 times better than the traditional IFN-2a [74]. It is indeed significant to observe that, EV71 inhibit IFN-mediated phosphorylation of STAT1, STAT2, JAK1 and Tyk2 via reducing IFNAR1 through EV71 2Apro [75]. Another study found that IFN-induced IRF1 transactivation is blocked by EV71. However, there was no change in the expression of IFN- receptors. Lack of type I and type II IFN receptors makes mice more likely to die after contracting EV71 [76]. It has also been revealed that a number of EV71 infection targets including RIG-1, MDA5, MAVS, TLR9, TLR7 and miR-146a, interact with type I IFN responses. As a result, solid evidence points to a crucial part IFN-mediated signaling pathways play in host innate immunity to EV71.

5.6. EV71 Interacts with IRF Signaling

Type I IFNs and IFN-inducible genes are crucial components of the immune system's response to pathogen-derived danger signals [77]. It has been shown that EV71 3Cpro inhibits RIG-I-mediated IRF3 activation and IFN-α/production. Similar to this, EV71 deactivates IRF3 and severely reduces gene expression induced by IFN [78]. On the other hand, study found that following EV71 infection to HT-29 cells, IRF3 activated and translocated into the nucleus. In addition, it has been suggested that IRF7 rather than IRF3 mediates cleavage of EV71-encoded 3Cpro. These contradictions need to be explained [79].

5.7. EV71 Triggers NF-κB Signaling

A protein complex called NF-κB regulates the transcription of several genes involved in immunological responses, cell proliferation, differentiation etc. Additionally, through promoting the expression of type I IFN, proinflammatory cytokines, NF-κB contributes significantly to host antiviral response [80, 81]. However, it has been noted that the EV71 2Cpro protein inhibits NF-κB activation and promotes virus replication. IKK and IBs

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kinase domains are directly contacted by the 2Cpro protein, which inhibits their phosphorylation. As a result, viral replication is boosted up and NF-κB activation decreases [82]. Evidence suggests that NF-κB signaling is necessary for respective virus replication and the inflammatory reactions [83]. As a result, NF-κB signalling is crucial in the inflammatory response that EV71 causes and offers a possible antiviral approach (Figure 4).

5.8. EV71 Interacts with TLR Signaling

Recent findings have provided strong evidence of activation of particular TLR mediates the EV induced innate immune response [10, 11]. There are 13 mammalian TLR are identified which expressed on the cell surface or in the endoplasmic reticulum. TLR1,2,4,5,6 have been found on cell membrane and TLR3,7,8,9,10,11,12,13 have been found in endosomal compartments. Lipopolysaccharide, lipoprotein and flagellin are recognized by the TLR4/MD2 (myeloid differentiation) complex, TLR1/6, TLR2 and TLR5 to activate NF-B and cause the generation of Type -I interferon. TLR7/8 recognizes the single stranded RNA of RNA virus [84]. TLR3 recognizes the viral double stranded RNA and recruits TRIF to induce TRAF3 and activate the TBK1/IKKε complex [85]. It can phosphorylate IRF7 or IRF3 to stimulate the production of IFN-I after activating TBK1/IKK. NF-B is dimerized and enters the nucleus after activating the TAK1/TAB2/TAB3/TAB1 complex, which results in the production of proinflammatory cytokines [86].

Dendritic cells (DCs) are activated and matured by EV71 when TLR4 stimulates IB breakdown and NF-κB activation. According to Song et al., 2018 enhanced EV71 replication brought on by autophagy in 16HBE cells encourages endosome breakdown and suppresses the type I IFN response mediated by TLR7 [87, 88]. The ESCRT-0 complex and the sorting of membrane protein in endosomes are controlled by hepatocyte growth factor-regulated tyrosine kinase substrates (HRS). During EV71 infection, HRS promotes the production of proinflammatory cytokines and interferons through the TLR7/NF-κB/p38 and TLR7/NF-B/IRF3 signaling pathways, which triggers inflammatory immune responses [89]. In contrast, TLR2, TLR7 and TLR8 mRNA were found to be upregulated inEV71-infection human primary monocytes derived macrophages at different time point and opposed to TLR3, TLR4, TLR6, TLR9 and TLR10 (MDMs). This finding implies that EV71 infection interacts with TLR signaling [90].

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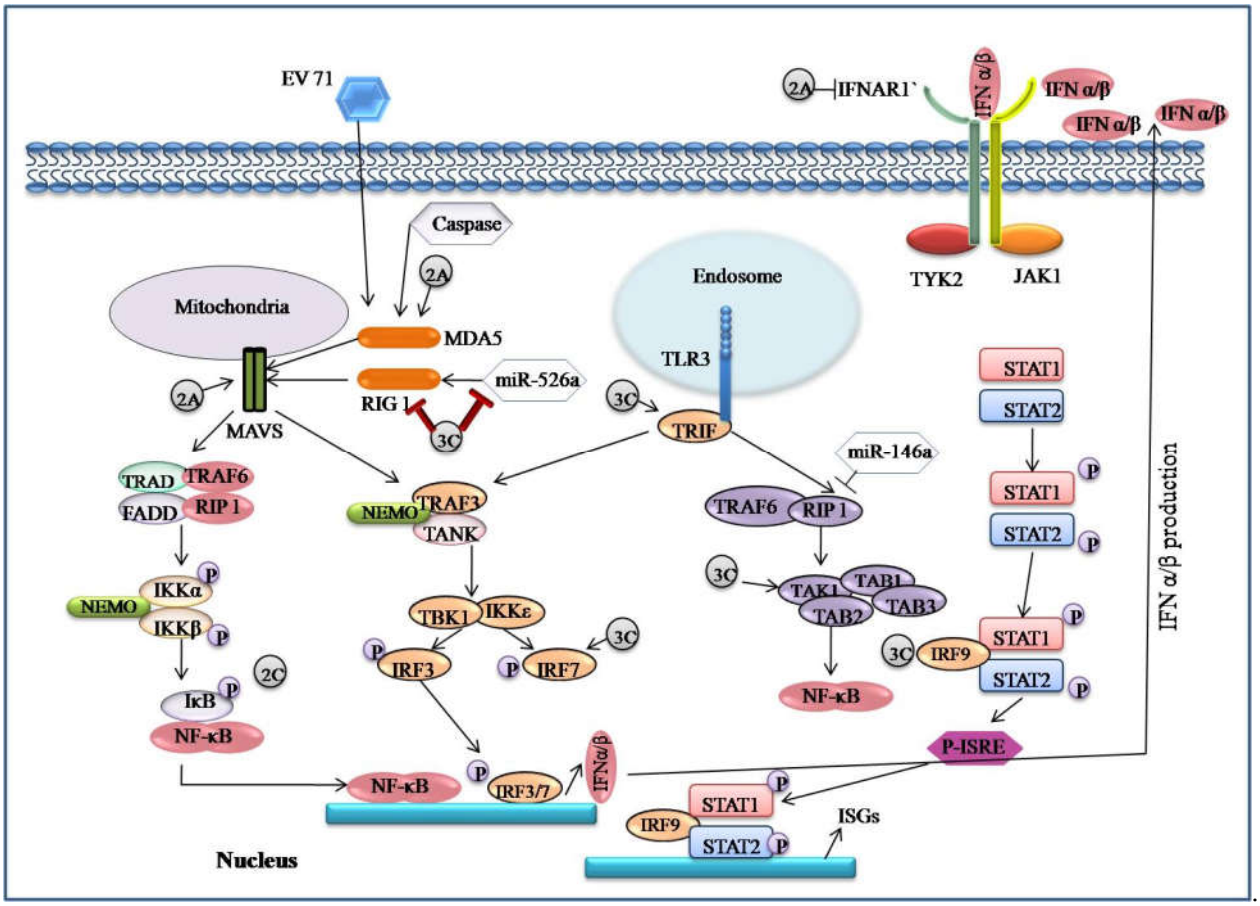

Figure 4. Evasion of PRRs-mediated signaling pathways by EV71. By means of the non-structural proteins 2A, 2C, and 3C, EV target the PRRs signaling pathway. 2A degrades IFNAR1 and cleaves MAVS and MDA5. RIG-1 and MAVS complex cleaves by 3c which also inactivates TRIF. The activation of RIG-1 bymir-526a inhibited by 3c and by promoting the expression of miR-146a, EV71 can also evade innate immunity.

6. Preventive and Therapeutic Strategies and Control of Ev71 (Pathological Perspective)

The infection increased through the EV71 outbreak has been developed by the various strategies of prevention and control of the disease outbreak. Different therapeutic and preventive strategies have been employed for the development of controlling processes for the disease development condition [91]. It is important to understand that the mixture of the natural products associated with the EV71 prevention and control the infection developed through the HFMD as well as other neurological or cardiovascular symptoms.

There are some natural components to support the context of the disease control based on the development of the clinical perspective to limit the effect of infection caused by the EV71. *Hydrolyzable ellagitannins* are associated with one of the most effective natural components to support the aspect of the therapeutic strategy development based on the presence of hexahydroxy diphenoyl unit to control infection. It has been observed that the components of hydrolyzable ellagitannins such as geraniin, chebulagic acid, and punicalagin are responsible for providing a prolonged survival time and reduced mortality rate by the EV71 infection development. It is important to understand that the hydrolyzable ellagitannins have been responsible for inhibiting the prospect of viral replication. On the other hand, chebulagic acid application as a natural therapeutic agent is responsible for restructuring the context of viral absorption within the host cell. Evidence for the inhibitory function of the EV71 has been associated with the application of chebulagic acid, geraniin, and punicalagin.

It has been observed that the alkaloids are responsible for restricting the elongation of the polyprotein of virus in the protein synthesis based on the interrupted procedure of the viral protein synthesis. However, synthetic antiviral components play an important role to prevent the infection. During the infection, pleconaril is one of the important synthetic antiviral components associated with the development of the strategy to prevent the mortality rate caused by the respective virus. Pleconaril, a synthetic component, is responsible for promoting the antiviral activity within the organism through the restricting action on the viral replication development. Imidazolidinone is a series of derivatives associated with the Pleconaril that is responsible for addressing the importance of the inhibitory action of the SCARB2 and PSGL-1. Evidence from Taiwan has provided the application of the imidazolidinone derivatives to control the function for the host-pathogen interaction [92].

Low cellular toxicity of the synthetic molecule peptide has been associated with the antiviral action against EV71. Rupintrivir is an important synthetic antiviral agent responsible for promoting the irreversible peptidomimetic inhibitors based on the restrictive procedure and inhibit the action of the viral replication [93]. Moreover, the synthetic component Rupintrivir is responsible for inhibiting the action of the VP1 to prohibit the action of the host-pathogen interaction and relative responses for the disease condition development. Sorafenib is responsible for providing post-infection condition by restrict viral RNA replication by blocking the signalling pathways such as p38 and EPK [94]. On the other hand, immunoglobulin treatment has been associated with the effective measure for neutralization of the antibodies based on the application of the immunoglobulin process such as IVIG-mediated treatment procedure. Clinical surveillance including preventive measures for EV71 infection can be helpful to formulate the understanding of the epidemic condition required for the health-conscious process in disease control.

7. Conclusion

The study has been associated with the identification of various factors including clinical, molecular, and immunological responses involved with the EV71. The pathological aspect including the factors associated with the virulence caused by the virus has been reflected. Moreover, identification of the epidemiological evidence based on the clinical and molecular aspects will provide the future scope for effective vaccine development as well as other sustainable preventive strategies.

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