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Enterovirus 71 infection and vaccines

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**K O R E A N
V A C C I N E
S O C I E T Y**

Hand, foot and mouth disease (HFMD) is a highly contagious viral infection affecting young children during the spring to fall seasons. Recently, serious outbreaks of HFMD were reported frequently in the Asia-Pacific region, including China and Korea. The symptoms of HFMD are usually mild, comprising fever, loss of appetite, and a rash with blisters, which do not need specific treatment. However, there are uncommon neurological or cardiac complications such as meningitis and acute flaccid paralysis that can be fatal. HFMD is most commonly caused by infection with coxsackievirus A16, and secondly by enterovirus 71 (EV71). Many other strains of coxsackievirus and enterovirus can also cause HFMD. Importantly, HFMD caused by EV71 tends to be associated with fatal complications. Therefore, there is an urgent need to protect against EV71 infection. Development of vaccines against EV71 would be the most effective approach to prevent EV71 outbreaks. Here, we summarize EV71 infection and development of vaccines, focusing on current scientific and clinical progress.

Keywords: Hand, foot and mouth disease, Enterovirus 71, Vaccine, VP1, Animal models

Hand, Foot and Mouth Disease

Hand, foot and mouth disease (HFMD) is generally considered to be a common exan-thematous illness mostly seen in children under 5 years old worldwide. HFMD is a fe-brile illness characterized by a maculopapular rash or blisters on the hands, feet, groin, and buttocks and is associated with painful ulcerative lesions of the mouth [1]. Infec-tion is usually self-limiting but is highly contagious. HFMD often occurs in small epi-demics in kindergartens or childcare centers since the virus is highly contagious [2]. The transmission route of HFMD is usually directly via the fecal-oral route or via naso-pharyngeal secretions, such as saliva, sputum, or from the runny nose of an infected person [3,4]. An indirect viral propagation path can be mediated by contaminated ma-terial that was touched by an infected person [3-5]. HFMD mainly affects infants and children because high incidence and severity was shown in young children under the age of five with weakened immune systems, rather than in adults [3,6]. In rare cases, HFMD develops to produce severe complications in the central nervous system (CNS), respiratory, and cardiovascular systems, including aseptic meningitis, cerebella ataxia, poliomyelitis-like paralysis, acute brainstem encephalitis, cardiopulmonary failure, and fulminant neurogenic pulmonary edema associated with high mortality [7]. Chil-dren who recover from brainstem encephalitis can be left with significant neurologic

after-effects. HFMD tends to occur in outbreaks during the warm temperatures of spring, summer, and fall, showing a strong seasonal pattern [3,6]. HFMD incidence is significantly higher when the humidity is increased, since infection with the causative virus increases sharply in hot and humid conditions [8].

HFMD is a common infection caused by a group of viruses. The two major causes of HFMD are coxsackievirus A16 (CVA16) and enterovirus 71 (EV71). EV71, belonging to the enterovirus A group from the *Picornaviridae* family [9], is the second most common cause of HFMD, after CVA16. Other kinds of enterovirus and other coxsackieviruses such as A5, A6, A7, A9, A10, B1, B2, B3, and B5 can also cause HFMD [3]. The incidence of HFMD caused by EV71 is less than that caused by CVA16; however, EV71 is a neurotropic virus, tends to be more severe, and is more likely to be associated with complications including neurological symptoms and heart disease such as myocarditis that can even be fatal [5,9].

Clinical Epidemiology

In April 1957, HFMD was first reported in eight cases of affected children in New Zealand. Subsequently, the main cause of HFMD outbreaks during the 1960s was CVA16 [10]. EV71 was first isolated from a 9-month-old child with encephalitis in 1969, in the United States. After the confirmation of HFMD caused by EV71, HFMD occurred sporadically throughout the world [11]. However, since 1997, HFMD has occurred mostly as epidemics in countries of the Asia-Pacific region [12]. The HFMD outbreaks caused by identified EV71 genotypes in the Asia-Pacific region are summarized in Fig. 1. HFMD in China was first reported in 1981 in Shanghai. In 2008, HFMD occurred in China, with 488,955 cases and 126 deaths reported [13]. Between 2008 and 2012, 7,200,092 cases of HFMD and 2,457 deaths were reported to the Chinese Center for Disease Control and Prevention. Therefore, HFMD in China has become a serious disease and one of the leading causes of death in

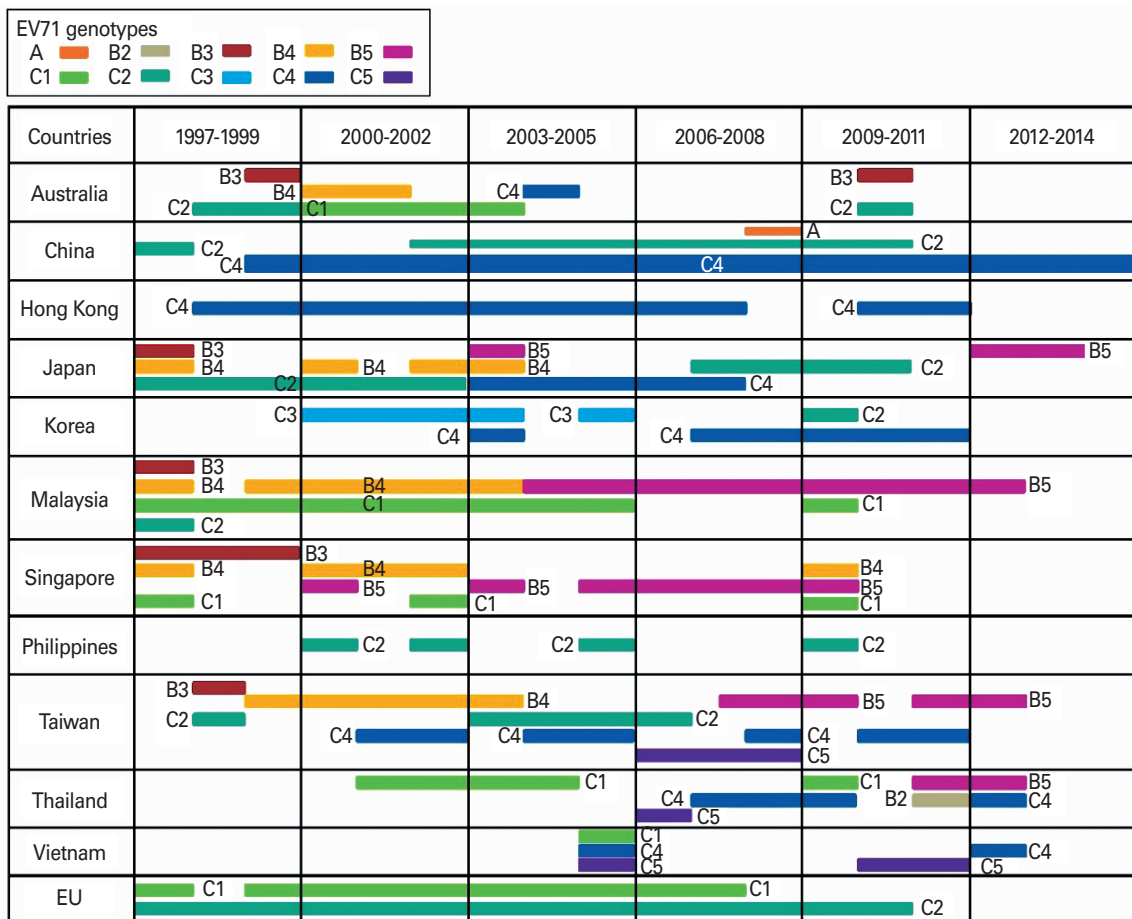**Fig. 1.** Enterovirus 71 (EV71) genotypes identified from outbreaks. The genotypes and sub-genotypes of EV71 strains identified from outbreaks since 1997 in each country of the Asia-Pacific region and from Europe (EU) are summarized. EU combines data from Austria, Germany, the Netherlands, and the United Kingdom. The data for 2012-2014 may be incomplete since recent statistics were not published in some cases.

children [14]. In Taiwan during 1998, 129,101 cases of HFMD were reported, and there were 405 serious cases of neurological complications such as meningitis and encephalitis, leading to 78 deaths [12]. In 2008, HFMD caused by EV71 resulted in 387 severe cases and 14 fatalities in Taiwan [12,15]. In Vietnam, HFMD caused by EV71 was first confirmed in 2003. From 2011 to 2012, over 200,000 patients with HFMD were hospitalized and 207 fatalities owing to HFMD were reported [16]. In South Korea, the first case of HFMD was reported in spring 2009 when the first death occurred [17]. In addition to these cases, many people suffered from HFMD in Japan in 1997 and 2000, in Singapore during 2000 and in Malaysia during 1997. Continuing to the present, HFMD has been continuously reported as occurring endemically in many Asia-Pacific countries [18]. C4 is the most prevalent EV71 genotype found recently in China, Hong Kong, Korea, and Vietnam, whereas B5 is the most common genotype circulating in Japan, Malaysia, and Taiwan.

Structure of the EV71 Virion

EV71 is a positive single-stranded RNA virus that belongs to the *Picornaviridae* family, genus Enterovirus, species Enterovirus A [19]. Human enterovirus serotypes include four species, enterovirus A, B, C, and D, which are distinguished by sequence differences, genome organization, and biological properties [20]. The EV71 particle is non-enveloped, icosahedral, and 20-30 nm in diameter (Fig. 2). The virus capsid contains a single-stranded, positive-sense, polyadenylated viral RNA. The RNA size is approximately 7.4 kb. The coding re-
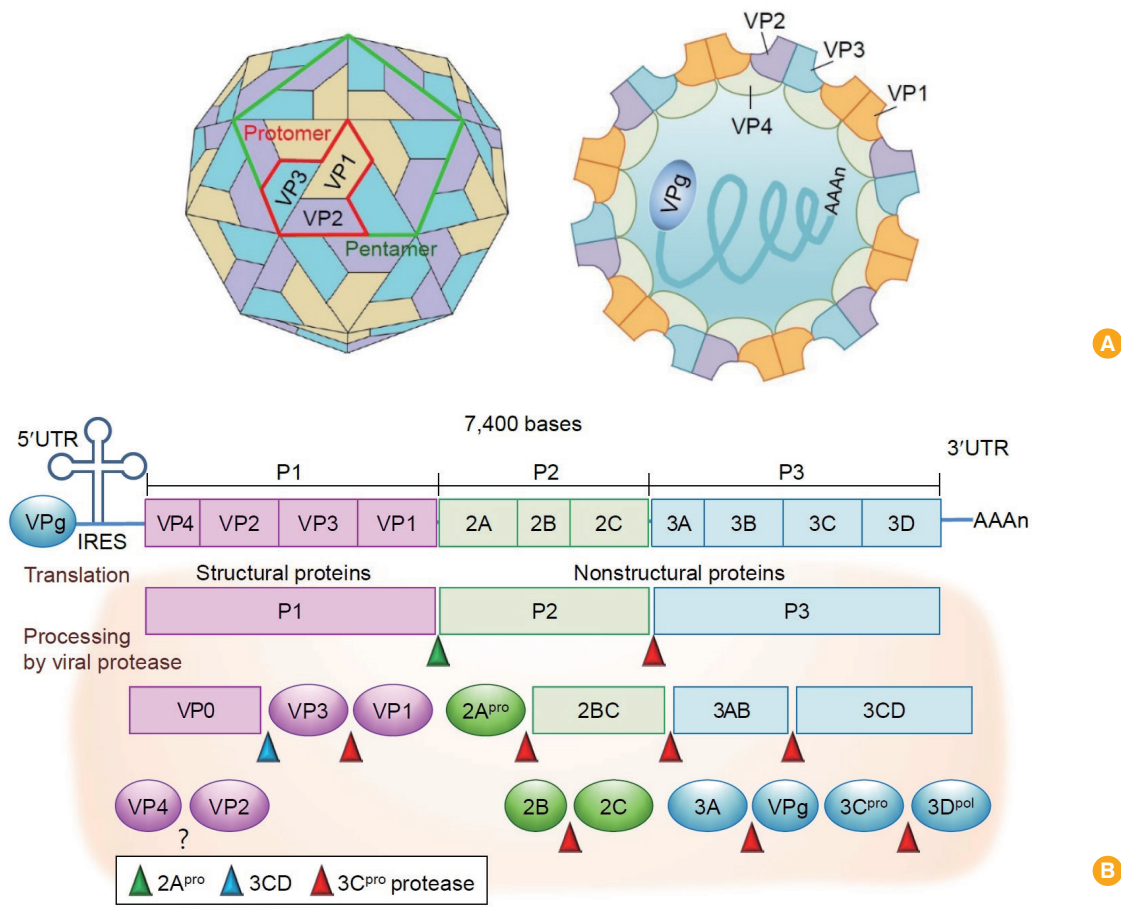
Fig. 2. Structure of the enterovirus 71 (EV71) virion. (A) EV71 is a non-enveloped and icosahedral particle with a diameter of 20-30 nm. The EV71 capsid consists of 60 copies of four different structural proteins (VP1-VP4). These four structural proteins assemble to form a protomer. Five protomers are assembled to form a pentamer, and 12 pentamers plus the viral genome form a virion. (B) The RNA genome of EV71 is approximately 7.4 kb, with an untranslated region (UTR) at the 5' and 3' ends of the genome. The 5'UTR contains an internal ribosomal entry site (IRES) for cap-independent translation. The 5'UTR is bound covalently to VPg (also known as viral protein 3B), and the 3'UTR includes a poly-A tail. The RNA is translated to a polyprotein that is sequentially cleaved by the viral 2A protease (2A[pro]), 3CD protease, and 3C protease (3C[pro]). Viral protein 3D has an RNA-dependent-RNA polymerase activity.

gion of EV71 is divided into three sub-regions, namely the P1, P2, and P3 regions. The P1 region encodes four structural viral proteins including VP1, VP2, VP3, and VP4. The P2 and P3 regions encode seven non-structural proteins including 2A-2C and 3A-3D that make up the proteases used in proteolytic cleavage to release structural proteins, and the RNA-dependent RNA polymerase [5,21]. The four structural proteins VP1 to VP4 assemble to form a protomer. Five protomers make up a pentamer, and 12 pentamers together form a virion enclosing the viral genome [22]. VP1, VP2, and VP3 are exposed on the capsid surface to the external environment and these proteins can therefore be easily targeted by the host immune response. In particular, VP1 contains the major neutralization epitopes, which can be used as biomarkers to assess vaccine potency. VP4 is present at an inner location within the capsid [23]. EV71 is classified into the three genotypes A, B, and C and then further divided into 11 sub-genotypes. Genotype A has the prototype strain (BrCr) only, whereas genotypes B and C have five sub-genotypes including B1 to B5, and C1 to C5, respectively. Such sub-classification of EV71 is dependent on sequence variations in VP1 [24]. Unlike eukaryotic mRNAs, all *Picornaviridae* viral genomes contain an internal ribosomal entry site (IRES) instead of a 5′-cap structure. Following entry of the virus particle into host cells and release of the viral genome from an endosome into the cytoplasm, viral RNA can be translated in an IRES-dependent manner. During translation and genome replication, the viruses require not only IRES-specific trans-acting factors (ITAFs) but also several host factors including T-cell-restricted intracellular antigen 1 (TIA-1) and TIA-1 related protein (TIAR) for effective viral replication [25]. The interaction of TIA-1 and TIAR with the 5′ untranslated region of the viral genome can positively enhance viral replication, although ITAFs usually regulate viral growth at the translational step [26].

EV71 Receptors on the Host Cell

Several viral receptors that are responsible for entry of EV71 into host cells have been characterized (Fig. 3). These receptors include human scavenger receptor B2 (hSCARB2), human P-selectin glycoprotein ligand 1 (PSGL-1), dendritic cell specific intercellular adhesion molecule-3 grabbing noninte-grin (DC-SIGN), annexin A2 (Anx2), heparan sulfate (HS), and sialylated glycan [27-32]. SCARB2 belongs to the scavenger receptor class B subfamily and is also known as lysosomal integral membrane protein II, LGP85, or CD36b like-2. SCARB2
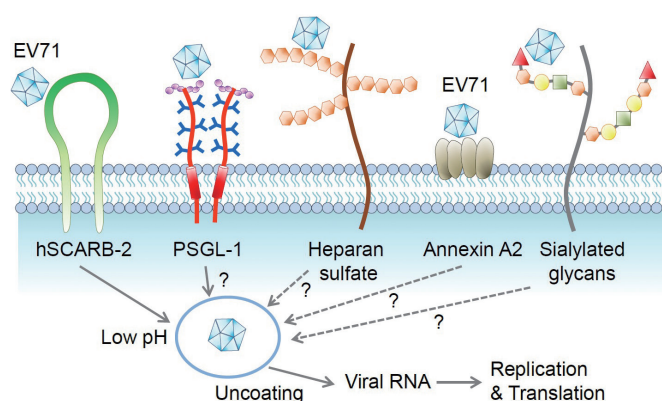

Fig. 3. Viral receptors for enterovirus 71 (EV71) on host cells. EV71 can bind human scavenger receptor B2 (hSCARB2), P-selectin glycoprotein ligand (PSGL-1), heparin sulfate, annexin A2, and sialylated glycans. EV71 enters host cells via receptor binding and becomes enclosed within an endosome having low internal pH. Following conformational deformation of the capsid, uncoated viral RNA is released into the cytoplasm, and protein translation and viral replication are initiated.

is known to participate in endocytosis, membrane transport, and reorganization of the endosomal/lysosomal compartments [33], and usually shuttles between the internal membrane and the plasma membrane. When it is present at the cell surface, SCARB2 is a type III double-transmembrane protein with a large extracellular domain and short cytoplasmic domains. Human SCARB2 on the cell surface binds to EV71, and then EV71 is internalized by the clathrin-mediated pathway. Mouse cells transformed with human SCARB2 are susceptible to all EV71 strains, and are capable of binding, internalization, and uncoating of the virus [34]. However, murine SCARB2 does not act as a receptor for EV71. Human SCARB2 is expressed in a variety of cell types, including neurons in the CNS, which may be involved in direct infection of the brain by EV71 [35]. PSGL-1 is a sialomucin-like protein and is generally expressed in leukocytes, with involvement in the tethering and rolling of leukocytes on the vascular endothelium during leukocyte infiltration. EV71 can infect T cells expressing PSGL-1, and infection of such cells can be inhibited by PSGL-1–specific antibodies [28]. After attachment, viral particles can be internalized by a caveolin-dependent pathway. However, PSGL-1 is unlikely to serve as the receptor on non-leukocytes such as neuronal, epithelial, and fibroblast cells since it is expressed primarily on leukocytes. Instead, leukocytes expressing PSGL-1 that become infected with EV71 might migrate and spread the virus into the CNS. PSGL-1 is not a receptor for all EV71 strains since approximately 80% of EV71 isolates are expected to lack binding to PSGL-1 based on se-

quence data [36]. DC-SIGN (also known as CD209) is a C-type lectin receptor present on the surface of both macrophages and dendritic cells that is involved in phagocytosis. Monocyte-derived dendritic cells can bind EV71 through DC-SIGN, and these virus particles can be transferred to susceptible cells to spread infection [32,37]. Anx2, a member of the annexin family, is a calcium-dependent phospholipid-binding protein that is involved in sorting of endosomes inside the cell and in anticoagulant reactions outside the cell. Anx2 can directly bind to VP1 of EV71 but not CVA16. However, subsequent entry and uncoating of EV71 have not yet been reported. HS is a linear polysaccharide found in all animal tissues, and two or three HS chains are attached to a proteoglycan (HSPG) in close proximity to the cell surface. HS can bind to various ligands and regulates a wide variety of biological processes. HS has been shown to bind a number of viruses including respiratory syncytial virus as well as EV71 [30]. Many glycan-binding proteins in pathogens recognize sialic acid or its modified forms expressed on the glycan chains of glycolipids and glycoproteins. Sialylated glycan is generally enriched in the epithelial cells of the respiratory and gastro-intestinal tracts. EV71 may use the sialylated glycan on intestinal epithelial cells as a receptor, although evidence for a direct interaction has not been reported [31].

EV71 binds viral receptors and enters the host cell via receptor-mediated endocytosis involving clathrin- or caveolin-mediated pathways [38]. Human SCARB2 on the cell surface binds to the EV71 virion and the SCARB2-EV71 complex is then internalized. When the virus-receptor complex reaches the endosome or lysosome, SCARB2 can initiate a conformational change at acidic pH leading to the uncoating of the virion. In contrast to poliovirus and group B coxsackieviruses, low pH is critical for EV71 uncoating. EV71 RNA is released into the cytoplasm following a series of structural changes in the viral capsids [5]. The viral RNA can be directly translated into a large polypeptide followed by prompt cleavage by the viral proteases (Fig. 2). Host cellular protein synthesis is shut down by the viral protease 2A, leaving viral protein synthesis unaffected. Viral replication takes place in the vesicle membrane complex by the RNA-dependent RNA polymerase 3Dpol. Following assembly of viral RNA and capsids, mature infectious virus particles are released when the infected host cell is lysed.

Animal Models for EV71 Infection

Although mechanisms for viral infection and host defense have been proposed, the details of the propagation process and pathology of EV71 have not been completely elucidated [39]. To obtain more knowledge about EV71 pathogenesis, various mouse and monkey animal models have been used. Neurological complications including ataxia, tremors, and flaccid paralysis are observed in EV71-infected cynomolgus monkeys, similar to the complications that occur in humans [40]. However, it is difficult to justify the use of monkeys as an infection model for EV71 infection owing to ethical and economic reasons. There have been reports of animal models of neonatal mice infected with mouse-adapted EV71 strains [41]. Unfortunately, virus replication in mice occurs mainly in muscle and fat cells, unlike in humans. Mice over 3-weeks-old do not show sensitivity to EV71; therefore, the suckling mouse model cannot be utilized to challenge with EV71 for a vaccine candidate. To allow established immunity in suckling mice, maternal immunization can be performed. The offspring of immunized female mice have high titers of maternal IgG specific to EV71, almost reaching the level seen for the immunized mother. These suckling mice when challenged with EV71 did not develop CNS complications and survived a lethal dose [42]. To mimic a weakened immune system, interferon (IFN) receptor-deficient mice with impaired viral defenses have been used. AG129 mice which lack receptors for both IFN-α/β and IFN-γ were susceptible to EV71 infection [43]. Treatment with neutralizing antibody for type I IFN reduces survival and increases disease progression following EV71 infection [44]. These results suggest that IFNs play an important role in antiviral defense against EV71 infection.

Recently, transgenic (Tg) mice with human SCARB2 were developed [45,46]. Three-week-old hSCARB2-Tg mice infected with EV71 via intracranial, intravenous, and intraperitoneal routes exhibited ataxia, paralysis, and death [45]. The pathological features in these mice were similar to those of EV71 encephalitis in humans. hSCARB2-Tg mice independently generated by another group were challenged with clinical isolates of EV71 and CVA16, resulting in an HFMD-like neurological syndrome and lethal paralysis [46]. Treatments with EV71-specific neutralizing antibody or pre-immunization with vaccine candidates were tested in the hSCARB2-Tg mice, and showed protection against subsequent lethal challenge with EV71 [46,47]. These results show that hSCARB2-Tg mice can be a useful model to assess anti-EV71 treatments. Human PSGL-1 as well as SCARB2 are thought to be functional receptors for EV71 in human cells [28,36]. When human PSGL-1 Tg mice were established, Tg expression of human PS-

GL-1 failed to enhance infectivity of EV71. However, human PSGL-1 expression did facilitate viral replication and symptom severity, but only at the earlier stages of infection. These results show that human PSGL-1 alone is not sufficient to mediate EV71 infection but may act as a cofactor for viral infection in mice in the early stages of EV71 infection.

Development of EV71 Vaccine Candidates

There have been several types of EV71 vaccine candidates, including attenuated strains, inactivated whole-virus, virus like particles (VLP), recombinant proteins, recombinant vectors, and peptide vaccines.

Recombinant proteins and synthetic peptides
Immunization with recombinant VP1 protein of EV71 expressed in *Escherichia coli*, yeast, or the baculovirus system can induce high levels of EV71 VP1-specific IgG antibody, and confer protection against EV71 infection [42,48-50]. Compared with the inactivated virus, recombinant VP1 elicited a lower titer of EV71-specific total IgG and provided protection only at a low challenge dose of EV71, although it can elicit similar levels of neutralizing antibody [48]. Immunization with SP70 synthetic peptide, which contains a neutralizing linear epitope from the EV71 VP1 capsid protein, could elicit a neutralizing antibody titer comparable to that obtained with a whole virion-immune serum [51]. Compared with the VP1 sequences of various sub-genotypes of EV71, the amino acid residues of epitope SP70 are highly conserved. However, synthetic immunogens require strong adjuvants.

Virus-like particles
VLPs for EV71, which resemble the natural virus capsid structure, have been produced and purified as potential vaccines [52,53]. Immunization with EV71 VLP was highly immunogenic and induced protective efficacy against lethal challenge in newborn mice. Based on this EV71 VLP technology, chimeric VLPs including combined SP70 epitopes of EV71 and CVA16 structural proteins or fusions of hepatitis B core antigen with SP70 epitopes of EV71 could elicit protective neutralizing antibodies in mice [54,55].

DNA vaccines and recombinant vector vaccines
DNA vaccines have also been tried for EV71. Immunization with DNA constructs containing the *VP1* gene of EV71 could elicit the production of VP1-specific IgG and neutralizing an-

tibodies against EV71 but showed low levels of antigenicity [56]. Maternal immunization with an attenuated *Salmonella enterica* serovar Typhimurium expressing the EV71 *VP1* gene conferred protection against lethal EV71 infection in the offspring [57]. Recombinant adenovirus with the EV71 P1 and 3CD genes can enhance neutralizing antibody and protective cellular immune responses to prevent EV71 infection [58]. Oral immunization using Tg tomato fruit expressing the EV71 VP1 protein can elicit both humoral and cellular immunity, including mucosal VP1-specific IgA antibody [59].

Live attenuated virus
Immunization of cynomolgus monkeys with an attenuated EV71 genotype A (BrCr) could produce high neutralization activity with cross-reactivity for other genotypes and confer protection against lethal challenge by virulent EV71 genotype A [60]. However, this strategy needs to overcome some safety issues, since the attenuated strain itself caused mild neurological symptoms and was still neurotropic when inoculated via the intravenous route. A high-fidelity variant of EV71 with the two amino acid modifications, L123F and G64R, in the viral 3D RNA polymerase exhibited an attenuated phenotype and showed potential as a live attenuated EV71 vaccine [61].

Inactivated whole virus
Among the various vaccine candidates, inactivated whole virus vaccines are the preparation of choice capable of fulfilling the demand for effective control. Development of inactivated whole-virus EV71 vaccines has progressed rapidly, inspired by previous developments in inactivated vaccines. Immunization with formalin inactivated EV71 strain can elicit high levels of virus-specific antibody including cross-neutralizing activity and protects the immunized host against lethal challenge with virulent EV71 in the murine model [48,62]. Based on successful pre-clinical work, phase I or phase II clinical trials of candidate inactivated EV71 vaccines were conducted. Vaccination induced significantly greater neutralizing antibody and specific T-cell responses in vaccinees without a marked inflammatory response [63]. Other inactivated EV71 vaccine candidates can elicit cross-neutralizing antibody responses against EV71 sub-genotypes B1, B4, B5, and C4A [64]. Recently, five inactivated EV71 vaccine candidates have been developed and evaluated in clinical trials (Table 1). EV71 vaccines developed by Beijing Vigoo Biological Co., Ltd., Sinovac Biotech Co., Ltd., and the Institute of Medical Biology, Chinese Academy of Medical Sciences (CAMS, Kunming Insti-

Table 1. Formalin-inactivated EV 71 vaccines tested in human clinical trials

Organizations	EV71 strain	Dosage (µg)	Population target	Sample size	Status	References
Sinovac Biotech Co., Ltd. (China)	C4a (FY7VP5 strain)	1	6-35 month children	10,245	Phase 3 completed, approved	NCT01507857
Beijing Vigoo Biological Co., Ltd. (China)	C4a (H07 strain)	0.8	6-35 month children	10,077	Phase 3 completed	NCT01508247
CAMS (China)	C4a (H07 strain)	0.25	6-71 month children	12,000	Phase 3 completed, approved	NCT01569581
NHRI (Taiwan)	B4	5 and 10	Adults	60	Phase 1 completed	NCT01268787
Inviragen (Singapore)	B2	0.6 and 3	Adults	36	Phase 1 completed	NCT01376479

EV71, enterovirus 71.

tute) are all inactivated whole-virus alum-adjuvant vaccines that use independently isolated C4 genotype virus as the vaccine strain. Successful phase III randomized, double-blinded, placebo controlled trials of EV71 vaccines in three companies have been completed. Phase III clinical trials of inactivated EV71 C4 vaccines have involved more than 30,000 infants and children [65-67]. The results have shown that the EV71 vaccines have good safety even in children and can prevent over 90% of EV71-associated HFMD or herpangina and 80% of other EV71-associated disease symptoms.

Approved EV71 Vaccines

In December 2015, the Food and Drug Adminstration (FDA) of China approved the first vaccine against EV71, made by the Institute of Medical Biology at the Chinese Academy of Medical Sciences. In January 2016, the Chinese FDA approved a second EV71 vaccine made by Sinovac Biotech. Approved inactivated whole EV71 vaccines are now in commercial production.

Anti-EV71 Medications besides Vaccines

EV71 has caused frequent outbreaks in the Asia-Pacific region during the past two decades and has been considered a significant public health problem for the young generation in the post-poliovirus eradication era. A variety of strategies to develop anti-EV71 agents such as small molecules or antibodies have been investigated [68].

Small molecules to inhibit EV71 infection

The first strategy is to target viral entry into host cells. Intervention at the virus entry step is an efficient anti-viral strategy. The conformational change of the VP1 protein during entry is a crucial step for initiation of successful viral infection. The epitopes recognized by EV71-specific neutralizing antibodies among viral capsid proteins were located mainly on the surface of VP1. Pleconaril and BPR0Z-194, pyridyl imidazolidinone compounds that bind VP1 to inhibit its conformational change, can interfere with EV71 replication [69,70]. Lactoferrin, an iron-binding glycoprotein from colostrum, can bind to VP1 and interfere with the infectivity of EV71 [71]. The second strategy is to target the viral protease. Proteolytic cleavages of the EV71 precursor polyprotein by 2A^{pro} and 3C^{pro} are critical for synthesis of functional viral proteins [5]. Rupintrivir has been shown to suppress human rhinovirus infection by preventing 3C^{pro} activity [72]. Rupintrivir can also inhibit EV71 replication by blocking EV71 3C^{pro} activity [73]. However, no specific inhibitor has been developed yet for blocking 2A^{pro} activity. The third strategy is to target viral replication. The EV71 RNA genome is replicated by 3D polymerase, which is an RNA-dependent RNA polymerase. Therefore, blockage of 3D polymerase can be a strategy to suppress EV71 replication. Nucleoside analogs such as ribavirin and the non-nucleoside analog DTriP-22 have been studied as EV71 polymerase inhibitors [74,75]. To inhibit EV71 replication, viral 3A and its precursor 3AB can be targeted since they play crucial roles for formation of the EV71 replication complex [76]. The fourth strategy is to target viral translation. Translation of EV71 RNA, which has no 5´-cap structure, is dependent on the IRES. Therefore, regulation of IRES utilization can be a strategy to control EV71 infection. Kaempferol, a flavonoid, has been shown to suppress EV71 replication via reduced IRES activity by modulating the composition of the IRES-specific transacting factors [77].

Alternative strategies: small interfering RNAs and monoclonal antibodies

Artificially generated small interfering RNAs (siRNAs) specific for viral gene sequences can efficiently inhibit viral gene expression. An siRNA targeting the 3D region has been shown to inhibit EV71 infection [78]. To inhibit EV71 infection, a num-

ber of EV71-neutralizing antibodies have been studied from various groups. The neutralizing epitopes mainly exist in the structural viral protein, VP1, and are also found in VP2 and VP3 [79]. Among them, two monoclonal antibodies with universal neutralizing capabilities, Mab51 and 10D3, have been reported. Mab51 recognizes a highly conserved linear epitope with an amino acid sequence of 215_KQEKD_219 in EV71 VP1 [80]. 10D3 recognizes a conformational epitope that lies on a highly conserved knob region in VP3 of EV71 [81]. However, although these antibodies have been reported to provide good prophylactic protection against a lethal dose of EV71 in mice, they do not offer any therapeutic effects following infection.

Conclusion

Severe neurological symptoms of HFMD caused by EV71 make HFMD infection a serious public health problem for young children in countries of the Asia-Pacific region. In the absence of effective treatment, the development of efficacious vaccines to prevent EV71 outbreaks has been a national priority in some countries. Currently there are two EV71 vaccines that have been approved and are commercially available in China. To enable the worldwide use of EV71 vaccine, the applicability against various EV71 pandemic strains needs to be demonstrated [79]. Therefore, time is required after the EV71 vaccines enter the market before effective protection against severe HFMD can be achieved. Current formalin-inactivated EV71 vaccines can protect against EV71 but not against CVA16 infection, which is the most common cause of HFMD. The development of a bivalent formalin-inactivated EV71/CVA16 vaccine or multivalent vaccine including other prevalent pathogenic enteroviruses should be the next step [82]. In the future, novel modes of administration need to be investigated to provide a safe needle-free EV71 vaccine for young children. To enhance immunity against infection at mucosal tissue barriers, mucosal delivery of vaccine antigens will be helpful to develop improved vaccines. For such vaccines, delivery technology allowing optimal mucosal delivery without additional immunostimulatory adjuvant materials need to be investigated.

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References

1. Goksugur N, Goksugur S. Images in clinical medicine. Hand, foot, and mouth disease. N Engl J Med 2010;362:e49.
2. Ooi MH, Wong SC, Lewthwaite P, Cardosa MJ, Solomon T. Clinical features, diagnosis, and management of enterovirus 71. Lancet Neurol 2010;9:1097-105.
3. Omana-Cepeda C, Martinez-Valverde A, del Mar Sabater-Recolons M, Jane-Salas E, Mari-Roig A, Lopez-Lopez J. A literature review and case report of hand, foot and mouth disease in an immunocompetent adult. BMC Res Notes 2016;9:165.
4. Zou XN, Zhang XZ, Wang B, Qiu YT. Etiologic and epidemiologic analysis of hand, foot, and mouth disease in Guangzhou city: a review of 4,753 cases. Braz J Infect Dis 2012;16:457-65.
5. Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, Ooi MH. Virology, epidemiology, pathogenesis, and control of enterovirus 71. Lancet Infect Dis 2010;10:778-90.
6. Kaminska K, Martinetti G, Lucchini R, Kaya G, Mainetti C. Coxsackievirus A6 and hand, foot and mouth disease: three case reports of familial child-to-immunocompetent adult transmission and a literature review. Case Rep Dermatol 2013;5:203-9.
7. Chumakov M, Voroshilova M, Shindarov L, et al. Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in Bulgaria. Arch Virol 1979;60:329-40.
8. Kim BI, Ki H, Park S, Cho E, Chun BC. Effect of climatic factors on hand, foot, and mouth disease in South Korea, 2010-2013. PLoS One 2016;11:e0157500.
9. Zhou F, Kong F, Wang B, McPhie K, Gilbert GL, Dwyer DE. Molecular characterization of enterovirus 71 and coxsackievirus A16 using the 5′ untranslated region and VP1 region. J Med Microbiol 2011;60(Pt 3):349-58.
10. Duff MF. Hand-foot-and-mouth syndrome in humans: coxackie A10 infections in New Zealand. Br Med J 1968;2:661-4.
11. Chong P, Liu CC, Chow YH, Chou AH, Klein M. Review of enterovirus 71 vaccines. Clin Infect Dis 2015;60:797-803.
12. Wang SM, Ho TS, Lin HC, Lei HY, Wang JR, Liu CC. Re-emerging of enterovirus 71 in Taiwan: the age impact on disease severity. Eur J Clin Microbiol Infect Dis 2012;31:1219-24.

13. Cho HK, Lee NY, Lee H, et al. Enterovirus 71-associated hand, foot and mouth diseases with neurologic symptoms, a university hospital experience in Korea, 2009. Korean J Pediatr 2010;53:639-43.

14. Chen B, Sumi A, Toyoda S, et al. Time series analysis of reported cases of hand, foot, and mouth disease from 2010 to 2013 in Wuhan, China. BMC Infect Dis 2015;15:495.

15. Huang SW, Hsu YW, Smith DJ, et al. Reemergence of enterovirus 71 in 2008 in taiwan: dynamics of genetic and antigenic evolution from 1998 to 2008. J Clin Microbiol 2009;47:3653-62.

16. Donato C, Hoi le T, Hoa NT, et al. Genetic characterization of Enterovirus 71 strains circulating in Vietnam in 2012. Virology 2016;495:1-9.

17. Kim SJ, Kim JH, Kang JH, et al. Risk factors for neurologic complications of hand, foot and mouth disease in the Republic of Korea, 2009. J Korean Med Sci 2013;28:120-7.

18. Yu H, Chen W, Chang H, et al. Genetic analysis of the VP1 region of enterovirus 71 reveals the emergence of genotype A in central China in 2008. Virus Genes 2010;41:1-4.

19. Wang X, Peng W, Ren J, et al. A sensor-adaptor mechanism for enterovirus uncoating from structures of EV71. Nat Struct Mol Biol 2012;19:424-9.

20. Fields BN, Knipe DM, Howley PM. Fields virology. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2007.

21. Huang SW, Cheng HL, Hsieh HY, et al. Mutations in the non-structural protein region contribute to intra-genotypic evolution of enterovirus 71. J Biomed Sci 2014;21:33.

22. Ma HC, Liu Y, Wang C, et al. An interaction between glutathione and the capsid is required for the morphogenesis of C-cluster enteroviruses. PLoS Pathog 2014;10:e1004052.

23. Rossmann MG, Arnold E, Erickson JW, et al. Structure of a human common cold virus and functional relationship to other picornaviruses. Nature 1985;317:145-53.

24. Tee KK, Lam TT, Chan YF, et al. Evolutionary genetics of human enterovirus 71: origin, population dynamics, natural selection, and seasonal periodicity of the VP1 gene. J Virol 2010;84:3339-50.

25. Lin JY, Li ML, Shih SR. Far upstream element binding protein 2 interacts with enterovirus 71 internal ribosomal entry site and negatively regulates viral translation. Nucleic Acids Res 2009;37:47-59.

26. Lin JY, Shih SR, Pan M, et al. hnRNP A1 interacts with the 5' untranslated regions of enterovirus 71 and Sindbis virus RNA and is required for viral replication. J Virol 2009;83:

6106-14.

27. Yamayoshi S, Yamashita Y, Li J, et al. Scavenger receptor B2 is a cellular receptor for enterovirus 71. Nat Med 2009; 15:798-801.

28. Nishimura Y, Shimojima M, Tano Y, Miyamura T, Wakita T, Shimizu H. Human P-selectin glycoprotein ligand-1 is a functional receptor for enterovirus 71. Nat Med 2009;15: 794-7.

29. Yang SL, Chou YT, Wu CN, Ho MS. Annexin II binds to capsid protein VP1 of enterovirus 71 and enhances viral infectivity. J Virol 2011;85:11809-20.

30. Tan CW, Poh CL, Sam IC, Chan YF. Enterovirus 71 uses cell surface heparan sulfate glycosaminoglycan as an attachment receptor. J Virol 2013;87:611-20.

31. Yang B, Chuang H, Yang KD. Sialylated glycans as receptor and inhibitor of enterovirus 71 infection to DLD-1 intestinal cells. Virol J 2009;6:141.

32. Ren XX, Ma L, Liu QW, et al. The molecule of DC-SIGN captures enterovirus 71 and confers dendritic cell-mediated viral trans-infection. Virol J 2014;11:47.

33. Yamayoshi S, Fujii K, Koike S. Receptors for enterovirus 71. Emerg Microbes Infect 2014;3:e53.

34. Yamayoshi S, Iizuka S, Yamashita T, et al. Human SCARB2-dependent infection by coxsackievirus A7, A14, and A16 and enterovirus 71. J Virol 2012;86:5686-96.

35. Patel KP, Bergelson JM. Receptors identified for hand, foot and mouth virus. Nat Med 2009;15:728-9.

36. Nishimura Y, Lee H, Hafenstein S, et al. Enterovirus 71 binding to PSGL-1 on leukocytes: VP1-145 acts as a molecular switch to control receptor interaction. PLoS Pathog 2013; 9:e1003511.

37. Lin YW, Wang SW, Tung YY, Chen SH. Enterovirus 71 infection of human dendritic cells. Exp Biol Med (Maywood) 2009;234:1166-73.

38. Chen P, Song Z, Qi Y, et al. Molecular determinants of enterovirus 71 viral entry: cleft around GLN-172 on VP1 protein interacts with variable region on scavenge receptor B 2. J Biol Chem 2012;287:6406-20.

39. Yi L, Lu J, Kung HF, He ML. The virology and developments toward control of human enterovirus 71. Crit Rev Microbiol 2011;37:313-27.

40. Liu L, Zhao H, Zhang Y, et al. Neonatal rhesus monkey is a potential animal model for studying pathogenesis of EV71 infection. Virology 2011;412:91-100.

41. Khong WX, Yan B, Yeo H, et al. A non-mouse-adapted enterovirus 71 (EV71) strain exhibits neurotropism, causing

neurological manifestations in a novel mouse model of EV71 infection. J Virol 2012;86:2121-31.

42. Kim YI, Song JH, Kwon BE, et al. Pros and cons of VP1-specific maternal IgG for the protection of Enterovirus 71 infection. Vaccine 2015;33:6604-10.

43. van den Broek MF, Muller U, Huang S, Aguet M, Zinkernagel RM. Antiviral defense in mice lacking both alpha/beta and gamma interferon receptors. J Virol 1995;69:4792-6.

44. Liu ML, Lee YP, Wang YF, et al. Type I interferons protect mice against enterovirus 71 infection. J Gen Virol 2005;86 (Pt 12):3263-9.

45. Fujii K, Nagata N, Sato Y, et al. Transgenic mouse model for the study of enterovirus 71 neuropathogenesis. Proc Natl Acad Sci U S A 2013;110:14753-8.

46. Lin YW, Yu SL, Shao HY, et al. Human SCARB2 transgenic mice as an infectious animal model for enterovirus 71. PLoS One 2013;8:e57591.

47. Chang HW, Lin YW, Ho HM, et al. Protective efficacy of VP1-specific neutralizing antibody associated with a reduction of viral load and pro-inflammatory cytokines in human SCARB2-transgenic mice. PLoS One 2013;8:e69858.

48. Wu CN, Lin YC, Fann C, Liao NS, Shih SR, Ho MS. Protection against lethal enterovirus 71 infection in newborn mice by passive immunization with subunit VP1 vaccines and inactivated virus. Vaccine 2001;20:895-904.

49. Wang M, Jiang S, Wang Y. Recombinant VP1 protein expressed in Pichia pastoris induces protective immune responses against EV71 in mice. Biochem Biophys Res Commun 2013;430:387-93.

50. Premanand B, Kiener TK, Meng T, et al. Induction of protective immune responses against EV71 in mice by baculovirus encoding a novel expression cassette for capsid protein VP1. Antiviral Res 2012;95:311-5.

51. Foo DG, Alonso S, Phoon MC, Ramachandran NP, Chow VT, Poh CL. Identification of neutralizing linear epitopes from the VP1 capsid protein of enterovirus 71 using synthetic peptides. Virus Res 2007;125:61-8.

52. Chung YC, Ho MS, Wu JC, et al. Immunization with virus-like particles of enterovirus 71 elicits potent immune responses and protects mice against lethal challenge. Vaccine 2008;26:1855-62.

53. Sun S, Gao F, Mao Q, et al. Immunogenicity and protective efficacy of an EV71 virus-like particle vaccine against lethal challenge in newborn mice. Hum Vaccin Immunother 2015;11:2406-13.

54. Zhao H, Li HY, Han JF, et al. Novel recombinant chimeric virus-like particle is immunogenic and protective against both enterovirus 71 and coxsackievirus A16 in mice. Sci Rep 2015;5:7878.

55. Ye X, Ku Z, Liu Q, et al. Chimeric virus-like particle vaccines displaying conserved enterovirus 71 epitopes elicit protective neutralizing antibodies in mice through divergent mechanisms. J Virol 2014;88:72-81.

56. Tung WS, Bakar SA, Sekawi Z, Rosli R. DNA vaccine constructs against enterovirus 71 elicit immune response in mice. Genet Vaccines Ther 2007;5:6.

57. Chiu CH, Chu C, He CC, Lin TY. Protection of neonatal mice from lethal enterovirus 71 infection by maternal immunization with attenuated Salmonella enterica serovar Typhimurium expressing VP1 of enterovirus 71. Microbes Infect 2006;8:1671-8.

58. Tsou YL, Lin YW, Shao HY, et al. Recombinant adeno-vaccine expressing enterovirus 71-like particles against hand, foot, and mouth disease. PLoS Negl Trop Dis 2015;9:e0003692.

59. Chen HF, Chang MH, Chiang BL, Jeng ST. Oral immunization of mice using transgenic tomato fruit expressing VP1 protein from enterovirus 71. Vaccine 2006;24:2944-51.

60. Arita M, Nagata N, Iwata N, et al. An attenuated strain of enterovirus 71 belonging to genotype a showed a broad spectrum of antigenicity with attenuated neurovirulence in cynomolgus monkeys. J Virol 2007;81:9386-95.

61. Meng T, Kwang J. Attenuation of human enterovirus 71 high-replication-fidelity variants in AG129 mice. J Virol 2014;88:5803-15.

62. Ong KC, Devi S, Cardosa MJ, Wong KT. Formaldehyde-inactivated whole-virus vaccine protects a murine model of enterovirus 71 encephalomyelitis against disease. J Virol 2010;84:661-5.

63. Liu L, Zhang Y, Wang J, et al. Study of the integrated immune response induced by an inactivated EV71 vaccine. PLoS One 2013;8:e54451.

64. Chou AH, Liu CC, Chang JY, et al. Formalin-inactivated EV71 vaccine candidate induced cross-neutralizing antibody against subgenotypes B1, B4, B5 and C4A in adult volunteers. PLoS One 2013;8:e79783.

65. Zhu FC, Meng FY, Li JX, et al. Efficacy, safety, and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2013; 381:2024-32.

66. Zhu F, Xu W, Xia J, et al. Efficacy, safety, and immunogenicity of an enterovirus 71 vaccine in China. N Engl J Med 2014;370:818-28.

67. Li R, Liu L, Mo Z, et al. An inactivated enterovirus 71 vaccine in healthy children. N Engl J Med 2014;370:829-37.

68. Kuo RL, Shih SR. Strategies to develop antivirals against enterovirus 71. Virol J 2013;10:28.

69. Zhang G, Zhou F, Gu B, et al. In vitro and in vivo evaluation of ribavirin and pleconaril antiviral activity against enterovirus 71 infection. Arch Virol 2012;157:669-79.

70. Shih SR, Tsai MC, Tseng SN, et al. Mutation in enterovirus 71 capsid protein VP1 confers resistance to the inhibitory effects of pyridyl imidazolidinone. Antimicrob Agents Chemother 2004;48:3523-9.

71. Weng TY, Chen LC, Shyu HW, et al. Lactoferrin inhibits enterovirus 71 infection by binding to VP1 protein and host cells. Antiviral Res 2005;67:31-7.

72. Matthews DA, Dragovich PS, Webber SE, et al. Structure-assisted design of mechanism-based irreversible inhibitors of human rhinovirus 3C protease with potent antiviral activity against multiple rhinovirus serotypes. Proc Natl Acad Sci U S A 1999;96:11000-7.

73. Lu G, Qi J, Chen Z, et al. Enterovirus 71 and coxsackievirus A16 3C proteases: binding to rupintrivir and their substrates and anti-hand, foot, and mouth disease virus drug design. J Virol 2011;85:10319-31.

74. Kishimoto C, Crumpacker CS, Abelmann WH. Ribavirin treatment of murine coxsackievirus B3 myocarditis with analyses of lymphocyte subsets. J Am Coll Cardiol 1988;12:1334-41.

75. Chen TC, Chang HY, Lin PF, et al. Novel antiviral agent DTriP-22 targets RNA-dependent RNA polymerase of enterovirus 71. Antimicrob Agents Chemother 2009;53:2740-7.

76. Arita M, Takebe Y, Wakita T, Shimizu H. A bifunctional anti-enterovirus compound that inhibits replication and the early stage of enterovirus 71 infection. J Gen Virol 2010;91:2734-44.

77. Tsai FJ, Lin CW, Lai CC, et al. Kaempferol inhibits enterovirus 71 replication and internal ribosome entry site (IRES) activity through FUBP and HNRP proteins. Food Chem 2011;128:312-22.

78. Tan EL, Tan TM, Tak Kwong Chow V, Poh CL. Inhibition of enterovirus 71 in virus-infected mice by RNA interference. Mol Ther 2007;15:1931-8.

79. Ng Q, He F, Kwang J. Recent progress towards novel EV71 anti-therapeutics and vaccines. Viruses 2015;7:6441-57.

80. Lim XF, Jia Q, Khong WX, et al. Characterization of an isotype-dependent monoclonal antibody against linear neutralizing epitope effective for prophylaxis of enterovirus 71 infection. PLoS One 2012;7:e29751.

81. Kiener TK, Jia Q, Meng T, Chow VT, Kwang J. A novel universal neutralizing monoclonal antibody against enterovirus 71 that targets the highly conserved "knob" region of VP3 protein. PLoS Negl Trop Dis 2014;8:e2895.

82. Klein MH. EV71 vaccines: a first step towards multivalent hand, foot and mouth disease vaccines. Expert Rev Vaccines 2015;14:337-40.