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Review 综 述

EV71 抗病毒药物及疫苗研究进展

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摘　要：手足口病(hand-foot and mouth disease，HFMD)作为全球关注的公共卫生疾病，亚洲地区频发，危害不容小觑。其传播途径广、传染性强、易引起并发症，致病株多样且易变异等特点，均为手足口病的治疗与防控带来挑战。肠道病毒 71 型(human enterovirus 71，EV71)作为手足口病主要的病原体之一，目前没有特效的抗病毒药物，故寻求合适的治疗药物极其重要。本文就 EV71 的抗病毒药物和疫苗研究进展进行总结，为手足口病的防控提供方向。

关键词：手足口病；肠道病毒 71 型；抗病毒药物；疫苗

Research progress of drugs and vaccines against enterovirus 71

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Abstract: Hand-foot and mouth disease (HFMD), as a disease of global concern, occurs frequently in Asia and its harm cannot be underestimated. The multiple transmission routes, strong infectivity, induction of complications, diverse pathogenic strains and easy variation bring challenges to the prevention and treatment of HFMD. Enterovirus 71 (EV71) is one of the main pathogens causing HFMD. Since there is no specific drug against EV71, it is of great significance to find appropriate

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therapeutic drugs. We summarize the research progress of drugs and vaccines against EV71, aiming to give insights into the prevention and control of HFMD.

Keywords: hand-foot and mouth disease; enterovirus 71; antiviral drugs; vaccines

手足口病(hand-foot and mouth disease，HFMD)是由肠道病毒71型(enterovirus 71，EV71)、柯萨奇病毒A组16型(coxsackievirus A16，CVA16)、柯萨奇A组10型(coxsackievirus A10，CVA10)以及柯萨奇A组6型(coxsackievirus A6，CVA6)等多种肠道病毒引发的传染病，流行同时病毒内部可通过亚基因型的改变和重组而变异[1–4]。该病人群普遍易感，患病人群中88.97%为5周岁及以下儿童，隐性感染率高，可通过飞沫、接触和饮食等方式传播，居住环境、生活方式、空间和气候等因素均影响发病率[5–7]。患者感染初期临床特征为发热、口腔溃疡、手脚部位会出现丘疹性水疱、斑疹等[8–10]。多数患儿7 d内自愈，但也有部分患儿会在几天后出现脑炎、脑膜炎、肺水肿和急性弛缓性麻痹等并发症，严重情况下会导致死亡[11]。

EV71是手足口病的主要病原体之一，1969年于美国加州福尼亚患有中枢神经系统疾病婴儿的粪便内首次分离出来，1998年于我国台湾地区首次流行[12–13]。EV71为单股正链RNA病毒，小核糖核酸病毒科，肠病毒属，正二十面体结构，直径约23–30 nm，有A、B、C这3种基因型[14–15]。P1、P2、P3是EV71的3种前体蛋白，可被自身酶系切割为结构蛋白(VP1、VP2、VP3和VP4)和非结构蛋白，其编码区基因重组会引起变种[16–17]。EV71具有嗜神经性，会在骨骼肌中复制，通过感染神经肌肉连接处的运动神经元，达到中枢神经系统，通过上下调节细胞因子、非蛋白编码基因的异常来诱导神经细胞凋亡和自噬[18–22]。侵入宿主后，先天免疫激活，病毒通过抑制TLR信号网络[23]、诱导宿主细胞代谢重编程[24]、刺激细胞因子信号转导蛋白(SOCS)的表达[25]、切割参与免疫的蛋白[26–27]等来规避免疫反应。由于目前仍无特效的抗病毒药物，为降低HFMD暴发的隐患，现针对EV71的药物研发及疫苗的研究进展综述如下[28]。

1 抗病毒药物

目前临床上通过静脉注射利巴韦林(ribavirin)，同时口服硝苯地平(nifedipine)、雷米普利(ramipril)来治疗由EV71引起的手足口病，患者7 d内完全康复[29–30]。利巴韦林可通过亚精胺-精胺N1-乙酰转移酶(SAT1)诱导多胺分解代谢，多胺耗竭限制病毒复制[31–32]。但是利巴韦林治疗过程中部分患者出现疲劳、贫血和头痛等不良反应[33]，因此研发特效的抗病毒药物是极其重要的(图1，表1)。

1.1 针对病毒进入的方式

1.1.1 靶向PSGL-1位点

P-选择素糖蛋白配体1 (P-selectin glycoprotein ligand 1，PSGL-1)是EV71的主要功能性受体，表达于骨髓细胞及白细胞表面，其N端残基能够介导EV71的结合和感染，基因多态性显著影响宿主对病毒的易感性[34]。研究发现，经横纹肌肉瘤(rhabdomyosarcoma，RD)细胞传代后的EV71 VP1残基突变株，仍与PSGL-1有极强结合能力[35]。因此靶向PSGL-1成为了抑制病毒结合的主要选择。Ren等[36]对PSGL-1介导的病毒入侵作用进行了阐述，并发现靶向PSGL-1的单克隆抗体KPL1可以阻断EV71与PSGL-1的结合，减少被感细胞的死亡，为靶向治疗EV71打下基础。

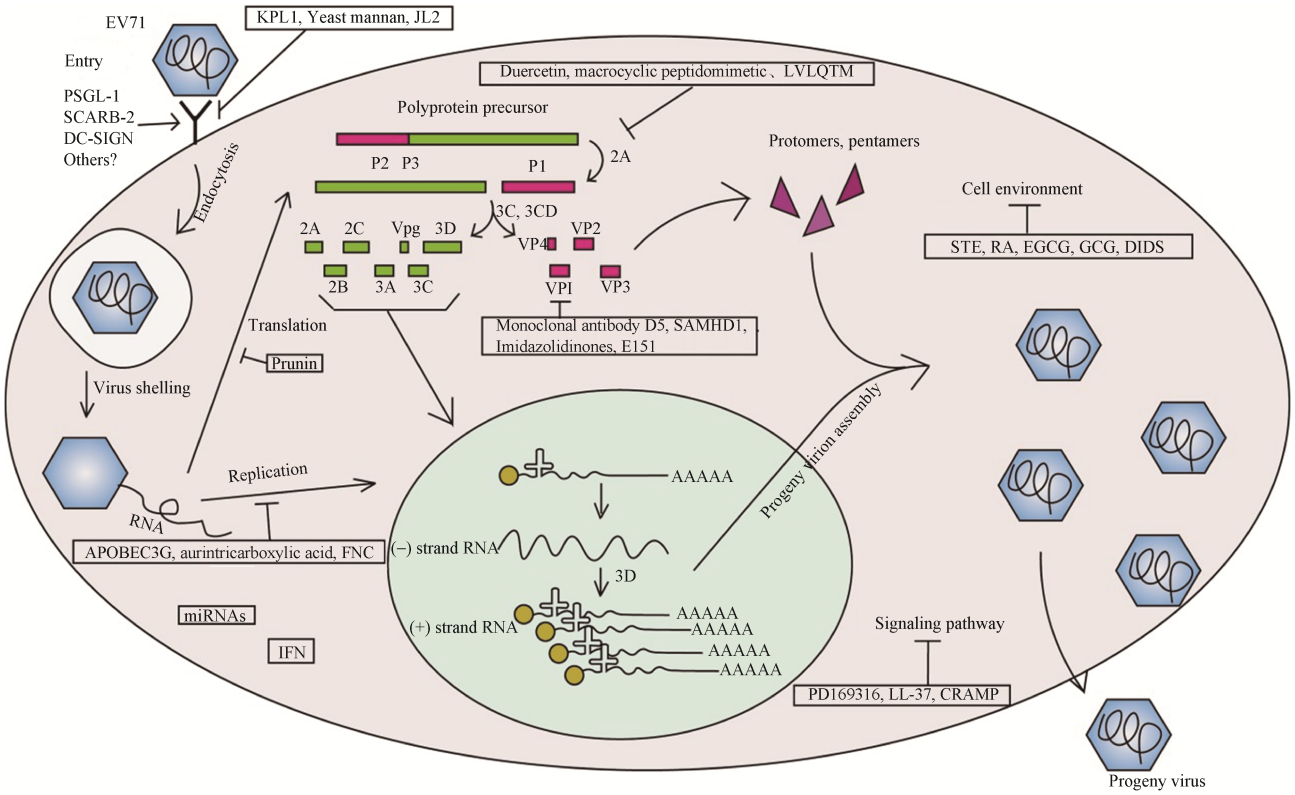

图 1　EV71 细胞内感染示意图和抗病毒药物概述

Figure 1　Schematic illustration of EV71 intracellular infection and summary of the antiviral agents.

1.1.2　靶向 DC-SIGN 位点

树突状细胞特异性细胞间黏附分子-3 结合非整合素(dendritic cell specific intercellular adhesion molecule-3 grabbing non integrin，DC-SIGN)作为 EV71 受体，与 PSGL-1 为协同作用，广泛分布于树突状细胞。研究发现，重症患者中 DC-SIGN 含量明显高于健康组，其单核苷酸多态性与 EV71 严重程度相关[37]。Ren 等[38]通过利用小干扰 RNA (small interfering RNA，siRNA)特异性敲除 DC-SIGN 后显著降低了病毒与细胞的结合率。酵母甘露聚糖也可阻断 DC-SIGN 的表达，降低细胞中 EV71 病毒量及 VP1 蛋白含量[39]。证实了 DC-SIGN 作为靶位点的有效性。

1.1.3　靶向 SCARB2 位点

清道夫受体 B2 (scavenger receptor class B member 2，SCARB2)作为 EV71 另一种功能性受体，广泛表达于人的各种组织，如胃底腺、肠黏膜上皮、支气管、肺细胞以及中枢神经系统的神经元等，其主要通过 152–163(α5)和 183–193(α7)螺旋与 EV71 VP1 的 GH 和 VP2 的 EF 环相互作用来介导病毒感染[40–41]。Zhang 等[42]构建出 SCARB2 的单克隆抗体 JL2，观察到其可与 SCARB2 的 2、5 和 14α 螺旋相互作用，阻止 EV71 与 SCARB2 的结合，从而抑制细胞病变。

1.2　针对病毒核酸翻译的方式

抑制 IRES 的活性：EV71 翻译的起始依赖内部核糖体进入位点(IRES)[43–44]。EV71 IRES 依赖性翻译需要 RNA 解旋酶 DDX3X，其通过与截短的真核翻译起始因子 4G (eIF4G)的相互作用，解开 IRES 结构域Ⅵ的二级结构促进核糖体进入[45]。因此靶向抑制 EV71 IRES 元件的活性，

表 1　体外和体内测试的抗 EV71 感染的抗病毒药物列表

Table 1　List of antivirals against EV71 infection tested *in vitro* and *in vivo*

Antivirals	Action site/mode	Strain type	*In vitro* cell type	*In vivo* mouse model	References
Blocking virus entry					
Monoclonal antibody KPL1	PSGL-1	EV71-G08-2	RD, Jurkat T		[36]
Yeast mannan	DC-SIGN	EV71 (HQ891927)	HEK293T		[39]
Monoclonal antibody JL2	The apical region of SCARB2	EV71 (0804232Y)	HEK293T		[42]
Inhibition of virus translation					
Prunin	EV71 IRES	EV71-41/H/B5/C4	RD	One-day-old suckling BALB/c mice	[47]
Inhibition of viral multiprotein processing					
Duercetin	The substrate binding pocket of EV71 $3C^{pro}$	EV71 (SK-EV006)	RD		[51]
Macrocyclic peptidomimetic	The substrate binding pocket of EV71 $3C^{pro}$	EV71 (Shenzhen/120F1/09)	RD		[52]
LVLQTM	The active site of EV71 $2A^{pro}$	EV71 (AEF32490)	HeLa		[58]
Inhibition of replication of virus					
Anti-$3D^{pol}$ monoclonal antibody	1–250 amino acids of EV71 $3D^{pol}$	EV71-MAV-VR	Vero	BALB/c aged 6–8 wks and pregnant ICR mice	[62]
APOBEC3G	EV71 $3D^{pol}$	EV71-H (VR-1432)	Vero		[63]
Aurintricarboxylic acid	EV71 $3D^{pol}$	EV71 (TW/4643/98)	Vero		[64]
FNC	EV71 $3D^{pol}$	EV71-CC063	RD	One-day-old ICR neonatal mice	[65]
Targeting virus capsid protein					
Monoclonal antibody D5 (mouse)	The surface exposed GH loop of VP1	EV71-G082	RD		[69]
Monoclonal antibody D5 (plant)	SP70 peptide of the VP1 on EV71	EV71 (MA V-W)	RD	Five-day-old ICR mice	[70]
SAMHD1	Domain in VP1 that binds to VP2 of EV71	EV71	HEK293T		[71]
Imidazolidinones (compound 27)	The hydrophobic pocket of VP1	EV71	RD		[72]
E151	The 5-fold axis of EV71	EV71-B2	RD	14-day-old AG129 mice	[73]
Regulation of host cell environment					
STE.		EV71 (CA-BrCr-70)	RD, Vero	Seven-day-old ICR mice	[75]
RA		EV71 (CA-BrCr-70)	RD		[76]
EGCG		EV71-BrCr	Vero		[77]
GCG		EV71-BrCr	Vero		[77]
DIDS	Blocking the current mediated by EV71 2B	EV71-SZ98	RD		[79]
Targeting MAPK signaling pathway					
PD169316		EV71-GDV103	RD, HeLa, Vero	Seven-day-old specific pathogen free mice	[81]
Cathelicidin (LL-37)	Enhance host immune response	EV71 (Fuyang 0805)	Vero	Newborn ICR mice (day 2–3, n=5)	[82]
Cathelicidin (CRAMP)	Enhance host immune response	EV71 (Fuyang 0805)	Vero	Newborn ICR mice (day 2–3, n=5)	[82]
Other					
Rheum emodin	Regulating CDK2 and cyclin A2 expression	EV71 (Changchun077)	Vero		[94]
Allophycocyanin		EV71-2231-TW	RD		[95]
Curcumin	Inhibit PKC δ phosphorylation	EV71 (Tainan/4643/98)	HT29		[96]
Ginsenoside Rb1	Stimulating immune response	EV71-695F	RD	Two-day-old suckling mice	[97]
PML	EV71 VP1	EV71-BrCr-TR	Vero, RD, HeLa	Three-day-old ICR mice	[98]
Acarbose	Blocking EV71 surface receptor binding sites	EV71-SK-EV006	DLD1, FHC CCD18-Co	One-day-old ICR suckling mice	[99]

RD: rhabdomyosarcoma cells; Vero: African green monkey kidney cells; HeLa: henrietta lacks cells; SK-N-SH: Henrietta Lacks cells; Jurkat: human T lymphocytes cells; HEK293 T: human embryonic kidney cells; HT29*:* human colon cancer cell; DLD1: human colorectal adenocarcinoma epithelial cells; FHC: human normal colorectal mucosa cells; CCD18-Co: normal human colon fibroblast adherent cells.

可以抑制 EV71 RNA 翻译的起始，阻碍蛋白合成，从而抑制成熟病毒的产生以及装配[46]。

Gunaseelan 等[47]筛选类黄酮化合物文库，发现樱桃苷(prunin)有效降低EV71感染BALB/c小鼠的临床症状和死亡率，明显降低 RD 细胞中 EV71 RNA 的含量，半数有效浓度(EC_{50})=0.115 3 μmol/L，半数致死浓度(CC_{50})=2.715 μmol/L。发现 EV71 经 prunin 处理连续传代后于第 13 代产生了抗性突变体，该突变允许其通过差异调节 IRES 反式作用因子 Sam68 和核内不均一性核糖核蛋白 K (hnRNP K)的募集来克服抗病毒作用，但该突变体可用第二药物进行治疗。这些研究确立了 prunin 可作为 EV71 治疗剂进一步开发为候选药物。

1.3 针对病毒多蛋白加工的方式

1.3.1 抑制 $3C^{pro}$ 的活性

EV71 3C 蛋白是半胱氨酸蛋白酶，通过蛋白加工、切割宿主蛋白来促进病毒复制、抑制 I 型干扰素反应来逃避先天免疫、激活 caspase 诱导宿主细胞凋亡等，其基因多态性与临床严重程度和病毒复制有关[48]。此前发现 EV71 $3C^{pro}$ 可通过切割端粒结合蛋白 PinX1 促进细胞凋亡[49]。近年发现 EV71 感染或异位表达 $3C^{pro}$ 可切割核内不均一性核糖核蛋白 A1 (hnRNP A1)，消除其与 Apaf-1 IRES 的结合，导致 Apaf-1 的 IRES 依赖性合成、caspase-3 的激活与细胞凋亡，进而释放病毒颗粒[50]。这些结果证实了 EV71 $3C^{pro}$ 在病毒增殖过程中的重要性以及其作为靶位点的有效性。

Yao 等[51]发现槲皮素(duercetin)可以插入 EV71 $3C^{pro}$ 的底物结合区域中，阻断底物识别，抑制 EV71 $3C^{pro}$ 的活性，但并不影响蛋白酶 $2A^{pro}$ 或 RNA 聚合酶 $3D^{pol}$ 的活性，在 RD 细胞中，EC_{50}=12.1 μmol/L，CC_{50}>200.0 μmol/L。此外 Li 等[52]就针对 EV71 $3C^{pro}$ 的晶体结构，用山口酯化反应代替典型的钌催化的烯烃复分解反应，设计出了具有确定构象的大环拟肽(macrocyclic peptidomimetic)，此新型药物可准确靶向于 $3C^{pro}$，抑制其活性，EC_{50}=4.5 μmol/L。

1.3.2 抑制 $2A^{pro}$ 的活性

EV71 2A 蛋白是含有 150 个氨基酸残基，具有半胱氨酸蛋白酶活性的蛋白酶，主要参与多蛋白的加工、抑制宿主蛋白合成、逃避先天免疫和诱导细胞死亡等[53–54]。$2A^{pro}$ 可诱导 TXNIP 介导的凋亡，调节 METTL3 的亚细胞位置来放大其自身基因表达的内部机制，参与病毒 RNA 的修饰[55–56]；还可通过切割 eIF4GI 来诱导非典型应激颗粒 aSG 的形成，以隔离细胞的基因，促进病毒的翻译[57]。研究发现，六氨基酸肽 LVLQTM 是 EV71 $2A^{pro}$ 的有效底物类似物，可与其直接相互作用，结合于 $2A^{pro}$ 的活性位点，抑制 $2A^{pro}$ 的 eIF4G 切割活性以及细胞中的 EV71 的复制，是直接靶向 $2A^{pro}$、抑制 EV71 复制的一个有效选择[58]。

1.4 针对病毒核酸复制的方式

抑制 $3D^{pol}$ 的活性：病毒侵入宿主细胞后，以正链 RNA 作为模板，并在病毒自身 RNA 聚合酶的指导下合成负链 RNA 进行扩增，过程主要由 RNA 依赖的 RNA 聚合酶($3D^{pol}$)指导[59–60]。EV71 $3D^{pol}$ 也参与 caspase 的激活，抑制 MDA-5 介导干扰素 β (interferon β，IFN-β)启动子的激活，具有拮抗宿主的作用[61]。

Li 等[62]将 $3D^{pol}$ 作为抗病毒研究的靶位点，构建出该位点的单克隆抗体(3A12 和 2A10)，直接干扰 $3D^{pol}$ 活性，显著抑制了病毒在体外的复制，且在应用浓度下细胞损伤小。Wang 等[63]发现胞苷脱氨酶(APOBEC3G，A3G)不仅能够抑制乙型肝炎病毒和丙型肝炎病毒复制，也可以与 EV71 $3D^{pol}$ 及病毒 RNA 相互作用，包装到子代病毒中以降低其传染性，其异位表达抑制了

EV71 的复制。除金三羧酸(aurintricarboxylic acid)[64]外，Xu 等[65]发现抑制艾滋病的小核苷类似物抑制剂 FNC 在 EV71 和 CA16 感染新生小鼠模型中，每 2 天以 1 mg/kg 体重进行 FNC 治疗，成功保护小鼠免受 EV71 和 CA16 病毒的致命攻击，并降低了各种组织中的病毒载量，EC_{50}=0.016 87 μmol/L，CC_{50}=3.238 μmol/L。

1.5 针对病毒衣壳蛋白的方式

结构蛋白 VP1 是 EV71 的衣壳蛋白，决定着病毒的基因型，极易发生氨基酸序列改变，是病毒的毒力决定簇，也是导致患者出现肺水肿的直接原因[66–67]。当 VP1 氨基酸重组时会出现病毒复制以及神经细胞自噬水平的不同[68]。鼠抗 EV71 单克隆抗体 D5，其可与 VP1 GH 环上的 SP70 肽特异性结合，阻断病毒附着和内化，以二价结合模式稳定病毒，有效中和 EV71 感染，半数抑制浓度 IC_{50}=0.324 μg/mL[69]。在此基础上以烟草花叶作为抗体生产系统，构建出 D5 单克隆抗体也可直接靶向表位 VP1 GH 环的 SP70 肽，中和 EV71，IC_{50}=1.53 μg/mL，这有效降低了药物研发成本[70]。

组氨酸-天冬氨酸结构域蛋白 1 (SAMHD1)是宿主限制性因子，可竞争作用于 EV71 VP1 与 VP2 的结合位点，阻断病毒颗粒的组装[71]。咪唑烷酮(imidazolidinones)作为杂环化合物，其化合物 27 不仅对 HIV 有效，还可以通过靶向 EV71 衣壳蛋白 VP1，抑制病毒吸附和 RNA 脱膜[72]。磺化偶氮染料-精黑 BN (E151)作为食品添加剂，可与 EV71 VP1 形成 5 重轴顶点相互作用，阻止病毒进入，在附着后阶段显著抑制 EV71，IC_{50}=10.10 μmol/L[73]。

1.6 针对宿主细胞环境的方式

1.6.1 抗氧化剂

氧化还原稳态是决定传染病预后的重要宿主因素，应激能够促进病毒复制。EV71 感染细胞上调了线粒体的生物生成，子代病毒诱导宿主细胞氧化应激促进病毒复制，因此抑制活性氧(ROS)也成为抗病毒复制的有效方法。

荆芥(schizonepeta tenuifolia Briq.，STE)是一种天然唇形科植物，目前用于银翘汤中治疗轻症疾病[74]。Chen 等发现 STE 不仅能够减少病毒的吸附和入侵，抑制 EV71 2A 蛋白酶切割 eIF4G，还可以抑制病毒诱导活性氧(ROS)形成以及核内不均一核糖核蛋白 A1 (hnRNP A1)从细胞核重新定位到细胞质，减少细胞病变效应，可作为保健食品或者潜在抗病毒药物进行开发[75]。

迷迭香酸(rosmarinic acid，RA)是山茱萸提取物(melissaofficinalis，MO)，其抗病毒作用机制与 STE 类似，通过抑制 ROS 介导的 p38 激酶激活，以及诸如 hnRNP A1 易位和 EPS15 等下游分子调节 EV71 感染细胞的膜转运过程，降低 EV71 的感染，IC_{50}=45.92±1.05 μg/mL，可作为治疗和预防 EV71 感染的候选药物[76]。

绿茶中的天然抗氧化剂表没食子儿茶素没食子酸酯(epigallocatechin gallate，EGCG)和没食子儿茶素没食子酸酯(gallocatechin gallate，GCG)，可以减少 ROS 的产生，并且可逆转在葡萄糖-6-磷酸脱氢酶(G-6-PD)缺乏的细胞中 EV71 复制的增强作用[77]。

1.6.2 离子通道阻断剂

柯萨奇病毒 B 组三型(CVB3)的蛋白质 2B 可以使游离细胞溶质 Ca^{2+}的浓度增加，以此促进病毒的释放[78]。Xie 等[79]将 EV71 2B 蛋白与 CVB3 2B 蛋白进行基因序列比较，并通过亚细胞定位分析、双电极电压钳记录膜电流、离子置换实验等方法，发现 EV71 中的 2B 蛋白也具有离子通道属性，可介导氯离子依赖性电流，促进病毒释放。同时发现 DIDS (4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid)作为离子通道阻断剂，明显抑制 RD 细胞中 EV71 病毒的产生，细

胞病变效应也验证了这一结果，为抗病毒治疗提供新角度。

1.7 针对信号通路的疗法

靶向 MAPK 信号通路：EV71 感染后，丝裂原活化蛋白激酶(mitogen-activated protein kinase，MAPK)信号通路被激活，刺激炎性细胞因子的释放，有利于 EV71 的感染[80]。Zhang 等[81]发现，PD169316 作为 p38-MAPK 信号通路的特定 p38 抑制剂，不仅可抑制 EV71 复制，还减少了 EV71 诱导的细胞凋亡。动物实验表明，PD169316 能够减少组织损伤并抑制炎性细胞因子的释放，减轻由 EV71 引起的乳鼠疾病。

人 LL-37 和小鼠 CRAMP 是内源性抗菌多肽(cathelicidin)，对囊膜病毒具有杀灭作用。当新生 ICR 小鼠感染了非囊膜病毒 EV71 后不同组织中的 CRAMP 表达显著上调，LL-37 或 CRIMP 能够上调 IFN-β 的表达及 IRF3 的磷酸化，下调 IL-6 及 MAPK 的活性直接或间接抑制病毒复制，为抗 EV71 感染的肽药物开发提供了有效的候选药物[82]。

1.8 其他方式

1.8.1 微小核糖核酸(miRNAs)

miRNAs 作为细胞内各种信号通路重要的调节因子，参与基因的表达调控，在病毒感染过程中发挥着不可或缺的作用[83]。

Li 等[84]发现，EV71 感染会降低 miR-9 的表达，同时诱导促炎因子(TNF-α、IL-6 和 IL-1)和干扰素(IFN-a 和 IFN-b)的高表达。如果诱导 miR-9 的过表达就能够降低 VP1 蛋白的表达和促炎因子的释放，通过介导 RIG-I 信号通路的 NF-κB 活性在细胞和小鼠模型中发挥抗 EV71 作用。

Wang 等[85]发现，EV71 感染后，miR-30a 会被外泌体包裹，以将其功能转移至受体巨噬细胞，通过靶向髓样分化因子 88 (MyD88)抑制 I 型干扰素反应，促进病毒复制。miR-146a 也通过外泌体介导转移，抑制 I 型干扰素反应，促进 EV71 感染，为反向抗病毒研究提供新思路[83]。

除此之外，还有多种 miRNA 在 EV71 感染过程中发挥重要调控作用。如 EV71 通过上调 miRNA 启动子的甲基化抑制 miR-17-92 簇[86]。宿主 miR-494-3p 通过直接靶向 PTEN 促进 EV71 复制[87]。敲除 miR-876-5p 后可减少细胞中病毒 RNA，miR-27a 可通过靶向 EGFR mRNA，抑制病毒复制[88–89]。miR-103/miR-107 通过调节 SOCS3/STAT3 途径抑制肠病毒 71 复制并促进 I 型干扰素应答[90]。这些均证实了 miRNA 在抗病毒治疗过程中的重要性，以及其在病毒感染过程中的调控能力，由于 miRNA 靶向位点的多样、调节通路复杂、易降解等特性，采用 miRNA 进行 EV71 感染的治疗仍有许多困难需要攻克。

1.8.2 干扰素

干扰素(interferon，IFN)是免疫调节的重要物质，已经应用于肿瘤及其他疾病的临床治疗。EV71 侵入宿主细胞后，单核细胞和 T 淋巴细胞便会分泌干扰素发挥抗病毒效应[91]。Liu 等[92]通过体外实验发现，在小鼠接种 EV71 前注射试剂上调 I 型干扰素表达量，能够明显减少病毒含量，降低小鼠死亡率。Su 等[93]在新生 C57BL/6J 小鼠腹膜内注射重组 IFN-λ2 后发现其抑制 EV71 复制并保护小鼠免受病毒攻击。这些结果均证实了干扰素的抗病毒作用，合理地利用可以发挥最优的抗病毒效果。

1.8.3 天然物质

大黄素(rheum emodin)是从中药大黄中提取的一种活性成分，EV71 感染人肺成纤维细胞系 MRC5，经大黄素治疗后，病毒基因组水平降低了 5.34 倍，病毒蛋白表达降低了近 30 倍，EV71 毒力降低了 0.331 07 倍，显著减少了 MRC5 细胞在 S 期的细胞周期停滞，具有抗 EV71 的效果[94]。

别蓝藻素(allophycocyanin)是从螺旋藻中提取的一种红色荧光蛋白,能够在病毒作用宿主细胞前后发挥作用，降低病毒的 RNA 合成、抑制病毒复制、延缓凋亡进程和减少宿主细胞的病变，IC_{50}=(0.045±0.012) μmol/L[95]。

姜黄素(curcumin)作为抗癌活性物质，Huang 等[96]发现姜黄素并不是如此前所说通过调节病毒吸附或者凋亡来发挥抗病毒作用,而是通过降低蛋白激酶 Cδ (PKCδ)的磷酸化，减少肠道上皮细胞中的病毒翻译作用,来增加宿主细胞的生存能力，抑制肠道病毒感染。

人参皂苷 Rb1 (ginsenoside Rb1)是西洋参中含量最丰富的三萜皂苷，Kang 等[97]最新发现，Rb1 能够以剂量依赖的方式降低 EV71 感染 RD 细胞的 CPE (cytopathic effect)及病毒 VP1 的表达，有助于增强 I 型 IFN 的表达，在感染的乳鼠中表现出比广谱抗病毒药物更强的抗病毒活性,可以作为一种免疫增强剂，EC_{50}=27.64 μmol/L。

PML (polysaccharide from *Monostroma latissimum*)是在绿藻单基质中分离出的硫酸鼠李糖(sulfated rhamnan)，Wang 等[98]发现在病毒吸附前或期间，PML 可靶向衣壳蛋白 VP1 来抑制病毒复制，还可通过调节表皮生长因子受体(EGFR)/磷酸肌醇 3-激酶(PI3K)/蛋白激酶 B (Akt)途径的信号来抑制病毒吸附后感染的早期步骤，IC_{50}=(0.5±0.3) μg/mL，无细胞毒性。

Feng 等[99]给乳鼠口服易感染小鼠神经细胞的 GFP-EV71 后,检测病毒在体内的动态分布及 EV71 在体内从肠到外周组织运输的动态途径，发现阿卡波糖(acarbose)可能通过阻断 EV71 病毒粒子表面上的受体结合位点或抑制细胞表面上的各种乙醇受体来减少 EV71 从肠到全身的动态转移。阿卡波糖及其类似物可能是预防 EV71 感染的潜在药物。

2 EV71 疫苗发展现状

为防止手足口病流行,疫苗接种是目前有效预防疾病的唯一措施[100−101]。在面对疫苗株选取、中和抗体检测、疫苗抗原定量、动物模型和临床验证等难点，各类疫苗的研发从未停止。以下就灭活疫苗、病毒样颗粒疫苗和肽疫苗研究进展进行总结。

2.1 灭活全病毒疫苗

灭活全病毒疫苗是指通过一定手段处理病原体使其失活,得到的无免疫原性的病原体制备的疫苗。自 2015 年我国成功研制出 EV71 全病毒灭活疫苗，于 2016 年投入使用，该疫苗在后期临床调查中均表现出无毒性、有限期长、安全性高和免疫原性强等特点,接种后可刺激免疫系统,上调干扰素、白介素以及 IgG 等含量[100−102]。但是仍有部分问题如少许接种者出现发热、过敏性皮疹、接种 EV71 疫苗后仍发展为脑炎患者的现象，并且当应急接种时，针对 EV71 的中和抗体的短期动态变化是未知的[103−105]。

Fan 等[106]研发了一种二价灭活的 EV71/CA16 疫苗，通过皮内途径注射 BALB/c 小鼠，在接种疫苗的局部上皮组织中检测到免疫信号分子的 mRNA，同时免疫相关趋化因子、干扰素上调，28 d 进行二次免疫后，成年小鼠会引发中和抗体和特异性 T 细胞反应，后代小鼠具有抵御病毒的能力。

由于疫苗制备的相似性以及患病的重叠性，联合疫苗的想法也由此诞生,用一种疫苗预防两种疾病，甲型肝炎(HAV)疫苗是中国国家免疫计划的一部分，是联合疫苗优选。Yang 等[107]将 HAV 灭活疫苗和 EV71 灭活疫苗进行联合制备了 HAV-EV71 灭活疫苗，在单剂量接种后，大鼠未引发不良反应，且观察到双抗体的产生，3 次接种后体重等生理指标未发生变化，无明

显过敏反应。联合疫苗或将成为未来疫苗研发的新趋势。

2.2 病毒样颗粒疫苗

病毒样颗粒疫苗是指去除遗传物质，仅存在病毒衣壳的具有免疫原性的蛋白制备的疫苗，在免疫原性评估中引发了可与灭活疫苗媲美的高而持久的中和抗体反应[108–109]。此前 Wang 等[110]利用昆虫表达系统，构建出 gag-VP1 VLP 组装体，通过体外小鼠实验，发现实验小鼠的体液免疫以及细胞免疫应答水平均高于对照组，并从后代体内检测出 EV71 抗体。

最近 Luo 等[111]通过共表达 EV71 P1 (在多角体蛋白启动子下)和 3CD (在 CMV-IE 启动子下)蛋白的重组杆状病毒(Bac-P1-3CD)制备 EV71 病毒样颗粒及其嵌合体，构建出显示保守的柯萨奇病毒 A16 表位的嵌合 EV71-VLP，结果显示实验母鼠、用免疫小鼠的血清被动转移的新生小鼠完全免受致命 EV71 攻击时，部分免受致命的 CA16 感染。

为高效研发疫苗，Yang 等[112]研发了产率高、工艺简单的重组技术，其在毕赤酵母中构建和表达了 EV71 的 *P1* 和 *3C* 基因，基于密码子优化的 *P1* 和 *3C* 基因，EV71-VLPs 在毕赤酵母系统中高效表达，表达量达到 270 mg/L。这些发现为手足口病今后的预防提供了一些可行的治疗方案，对疫苗的完善提供了有价值的参考。

2.3 肽疫苗

除灭活疫苗、病毒样颗粒疫苗外，肽疫苗的研发也取得了可观进展。Lei 等[113]从患者体内分离出 EV71 菌株，从 VP1 蛋白截断的 20 种合成肽中，筛选出了免疫原性较强的 3 种肽(肽 2、肽 4 和肽 8)制备了肽疫苗，结果显示肽疫苗改善了炎症，降低了肌肉和小肠中的病毒颗粒水平，并保护脑组织免受 EV71 感染，虽然其免疫效果不如灭活疫苗，但损害较小，可用于研究抗 EV71 疫苗的具体机制。

此外 Liu 等利用融合 PCR 技术扩增了 EV71 衣壳蛋白(VP2 N 端 180 个氨基酸，VP3 N 端 120 个氨基酸，VP1 C 端 131 个氨基酸)的 DNA 片段，在 T7 启动子的控制下，将 3 个片段连接在一起，形成 48 kDA 融合蛋白；这种肽疫苗组装后类似病毒颗粒，接种后可观察新生小鼠的主动免疫，此外其诱导产生的特异性血清抗体可使患病小鼠对 EV71 产生有效抵抗，具有安全和生产优势[114]。但是即使有辅助剂，肽疫苗免疫效果仍较弱，为增强肽疫苗的免疫强度，克服其局限性，Kim 等利用纳米技术将 EV71-VP1 表位肽和间隔交联剂偶联到长链脂肪酸的 N 端，开发了一种在生理 pH 值(pH 7.4)下自组装成纳米纤维的肽两亲物 PA (peptide amphiphile)，结果显示 PA 组表现出比肽组更高的免疫反应，有效地增强了对 EV71 感染的免疫反应，克服了肽疫苗的局限性[115]。

3 展望

回顾手足口病暴发至今，随着致病机理的不断剖析，治疗手段也在不断完善。虽然目前仍未开发出特效抗病毒药物，但临床上已经通过广谱抗病毒药物联合对症治疗来对抗手足口病。EV71 灭活疫苗的成功研发、中小学对疾病预防的重视，大幅降低了手足口病的暴发几率。

但是仍有一些问题需要克服：(1) EV71 具有嗜神经性，机理并不清楚，严重程度下造成的神经系统疾病能否避免。(2) 包括严重急性呼吸系统综合征冠状病毒-2 (SARS-CoV-2)在内，均具有逃避先天免疫的能力，能否克服也是一个难点。(3) 病毒具有潜伏期，大多数发病时已经错过最佳治疗时期，如果能在病毒潜伏期就检测、杜绝病毒，不仅降低了治疗成本，也降低了对患者的损害。(4) 抗病毒药物是治疗的另一重要手段，能够从根本上扼杀病毒，因此其研发是必不

可少的。(5) 虽然已经批准3种灭活EV71疫苗，但大部分农村地区疫苗覆盖率较低，家长接种意愿不高，因此应该加大宣传力度，重视疾病防御[116]。

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