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乙型肝炎抗病毒药物研究进展

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摘要 乙型肝炎病毒(hepatitis B virus, HBV)在世界范围内流行, 能够引起急、慢性肝炎, 其中长期慢性感染可增加肝硬化及肝癌的发病风险. 抗病毒药物治疗是控制HBV慢性感染的有效方法. 目前应用于临床的抗HBV药物主要有两类: α干扰素和核苷(酸)类似物. 这两种药物虽然可以抑制病毒复制, 减轻或消除肝脏病理损害并有效阻止病情向肝硬化和肝癌发展, 但并不能有效地达到HBV表面抗原(HBV surface antigen, HBsAg)使其转阴并清除病毒共价闭合环状DNA(covalently closed circular DNA, cccDNA), 所以HBV慢性感染很难治愈. 近年来, 随着人们对HBV研究的深入, 针对其复杂的感染机制及病毒与宿主免疫系统之间相互作用的不同靶点药物被相继开发出来. 本文对近年来在病毒直接靶向和宿主靶向药物(一些目前正处于临床试验阶段), 以及免疫治疗药物研发方面所取得的进展进行综述. 此外, 还对高通量筛选技术(unbiased high-throughput screens)寻找新的抗病毒化合物, 以及老药新用的策略用于抑制HBV进行了探讨.

关键词 乙型肝炎病毒, 药物, 直接抗病毒药物, 免疫疗法, 药物筛选

乙型肝炎病毒(hepatitis B virus, HBV)是一种小的、有包膜的、部分双链DNA病毒, 属于嗜肝DNA病毒科[1]. 目前, 全世界约有3亿人感染HBV[2,3], 大多数成年人感染HBV后形成急性感染, 病毒被机体的免疫系统清除, 然而也有一部分成年人和超过半数以上的新生儿感染HBV后不能自愈, 逐渐转变成慢性乙型肝炎(chronic HBV infection, CHB). 慢性乙肝是引起病毒感染相关并发症如肝硬化和肝癌的主要原因, 每年死于这类并发症的约有700000人[2,4]. 尽管目前开发的疫苗能够有效抑制HBV的母婴传播, 但是对于已经感染HBV的患者来说相关治疗性药物的应用依然受限. 目前已经批准用于慢性HBV感染治疗的药物主要有两类: α干扰素类和核苷(酸)类似物[5,6]. 这两类药物均可以在一定程度上抑制HBV复制, 并且有极小的几率使HBV表面抗原(HBV surface antigen, HBsAg)转阴, 但很难彻底清除肝细胞内乙肝病毒的复制模板-共价闭合环状DNA(covalently closed circular DNA, cccDNA)[6,7]. 因此, 通过抑制或清除cccDNA以及阻断再感染来实现慢性乙肝的治愈具有重大意义.

在临床上, 慢性乙肝的治愈分为两个层面: (1) 功能性治愈. 在患者血清中检测不到HBsAg和HBV DNA, 同时伴有或不伴有乙肝表面抗体的血清学转换. 但HBV cccDNA可能在肝细胞中持续存在, 在完成有限治疗疗程后肝损伤消退、肝细胞癌(hepatocellular carcinoma, HCC)风险降低. (2) 完全治愈. 完全根除HBV, 包括肝内cccDNA和整合的HBV DNA[7~9]. 功能性治愈是乙型肝炎治疗所追求的目标, 但真正达到的比例非常低. 因此, 深入研究HBV的生活周期并开发针对

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HBV不同靶标的治疗新药具有重要意义. 本文就HBV生活周期及相关潜在药物靶点的最新研究策略进行综述. 这些靶点包括病毒自身组分(直接抗病毒药物)和病毒生活周期相关宿主因子(宿主靶向药物)以及参与免疫调节的相关因子[2,10,11].

HBV基因组全长约3.2 kb, 由一条长的(−)-DNA链和一条短的(+)-DNA链组成的环状部分双链DNA[12]. 完整的HBV病毒粒子为直径约42 nm的球型, 由外向内依次为囊膜、核衣壳及基因组. 囊膜主要由来源于宿主细胞的脂质成分及病毒的3种表面蛋白构成; 核衣壳呈二十面体对称结构, 主要成分为病毒的核心蛋白[1]. HBV具有独特的生活周期, 包含多个过程(图1). HBV侵入机体后, 首先通过病毒表面蛋白上的决定簇与肝细胞表面的硫酸乙酰肝素蛋白聚糖(heparan sulfate proteoglycans, HSPGs)通过低亲和力相互作用完成吸附过程[13,14]. 然后HBV 大表面蛋白L(large surface protein, L)的preS1结构域与肝脏特异性受体Na^+-牛磺胆酸盐转运蛋白(Na^+-taurocholate cotransporting polypeptide, NTCP)产生高亲和力相互作用, 以此完成病毒的感染进入过程[15,16]. NTCP属于溶质载体蛋白家族10成员1 (solute carrier family 10 member 1, SLC10A1), 仅在肝细胞的基底外侧膜和窦状膜上表达, 其在体内的生物学功能是将肠肝循环中缀合的胆汁盐(例如牛磺胆酸盐)转运到肝细胞[17]. NTCP在HBV的肝脏靶向性中发挥关键作用, 特异性介导HBV和丁型肝炎病毒(hepatitis D virus, HDV)感染肝细胞[16]. 然而, 过表达人NTCP的小鼠肝细胞并不具有HBV易感性[18], 而且具有相似高水平表达人NTCP的人肝癌细胞系HepG2-NTCP细胞的不同单克隆对HBV的易感性也不相同[19]. 这表明HBV的有效感染需要额外的细胞因子参与.

HBV囊膜与肝细胞膜融合后通过受体介导以及小窝蛋白和网格蛋白参与的内吞作用, 并由RAB5和RAB7介导的内涵体转运[20], 从而将核衣壳释放到细胞内[21]. 病毒核衣壳沿着微管迁移到细胞核外缘并在输入蛋白家族细胞转运受体(cellular transport receptors of the importin family)的介导下通过核孔进入细胞核[22,23]. 核孔蛋白153是一种通过输入蛋白β参与核转运的核篮中的蛋白质, 它能够通过强相互作用将病毒核衣壳保留在核孔复合物的核侧[24]. 核衣壳在核孔处分解后释放病毒的松弛环状DNA(relaxed circular DNA, rcDNA)至细胞核中. 在鸭HBV(duck HBV, DHBV)模型上显示, rcDNA可能在酪氨酰-DNA-磷酸二酯酶2(TDP2)修复酶的作用下[25~27], 经正链共价连接的病毒聚合酶(polymerase, P)和RNA引物的去除、负链末端冗余序列的切割、不完整正链的修复, 以及两条DNA链的连接等多个步骤转化为cccDNA. 但对于人来源的HBV, 即使在TDP2敲除的细胞中仍可以成功建立感染, 这也就预示着其他与TDP相关的蛋白可能参与了人来源HBV cccDNA形成这一过程[11,12,27]. HBV cccDNA在感染的肝细胞中与组蛋白和非组蛋白结合形成复合物并作为小染色体稳定存在. HBV cccDNA的转录由4个启动子(核心, pre-S1, pre-S2/S和X启动子)和2个增强子控制, 并在RNA聚合酶2作用下转录形成4种病毒mRNA: 包括分别编码核心(core)/前核心(pre-core)和P蛋白的pre-core RNA及前基因组RNA(pre-genomic RNA, pgRNA)(3.5 kb), 编码S蛋白的preS1 RNA(2.4 kb)和preS2/S RNA(2.1 kb), 以及编码HBx蛋白的X RNA(0.7 kb)[28]. 增强子I介导转录早期(HBx mRNA)的激活, 增强子II介导晚期转录本的产生[29].

HBV P蛋白是从pgRNA内部的一个AUG密码子开始翻译, 其他HBV蛋白的翻译则是由最靠近其mRNA 5′末端的起始密码子处开始[30]. P蛋白与pgRNA 5′端ε包装信号结合形成复合体, 并随之招募core蛋白多聚复合体, 从而起始核衣壳的形成[31]. 其中, 热休克蛋白90介导pgRNA和P蛋白之间的相互作用[32]; 丝氨酸精氨酸蛋白激酶可以与core蛋白的C末端结构域结合, 调节该蛋白的磷酸化以防止其自组装和非特异性RNA的包装[33]; 人胞苷脱氨酶APOBEC3G可通过与病毒P蛋白结合而掺入病毒颗粒中[34], 可能作为病毒复制的一个负调控因子发挥功能.

在病毒核衣壳内, P蛋白末端蛋白结构域作为启动pgRNA逆转录的引物将其逆转录为(−)-DNA, 同时伴随着P蛋白RNase H活性功能对模板pgRNA的降解[35]. 当到达pgRNA的5′末端时, P蛋白以保留下来的一段RNA寡聚体为引物开始合成(+)-DNA[36]. 由于未知的原因, (+)-DNA的合成并不完整, 长度大约只有基因组全长的一半, 从而导致环状部分双链rcDNA的形成[37]. 在L蛋白的调控下, 含有rcDNA的核衣壳或被转运至细胞核中扩增cccDNA池, 或被包装成子代病毒粒子释放至细胞外[38]. HBV病毒粒子的组装, 首先是核衣壳通过NEDD4和衔接相关蛋白复合物1亚基γ2(adaptor-related protein complex 1 subunit gamma 2, AP1G2)运输到多泡体(multivesicular bodies, MVBs)表面[39]. 然后通过转运需要的内体分选复合物(endosomal sorting complex re-

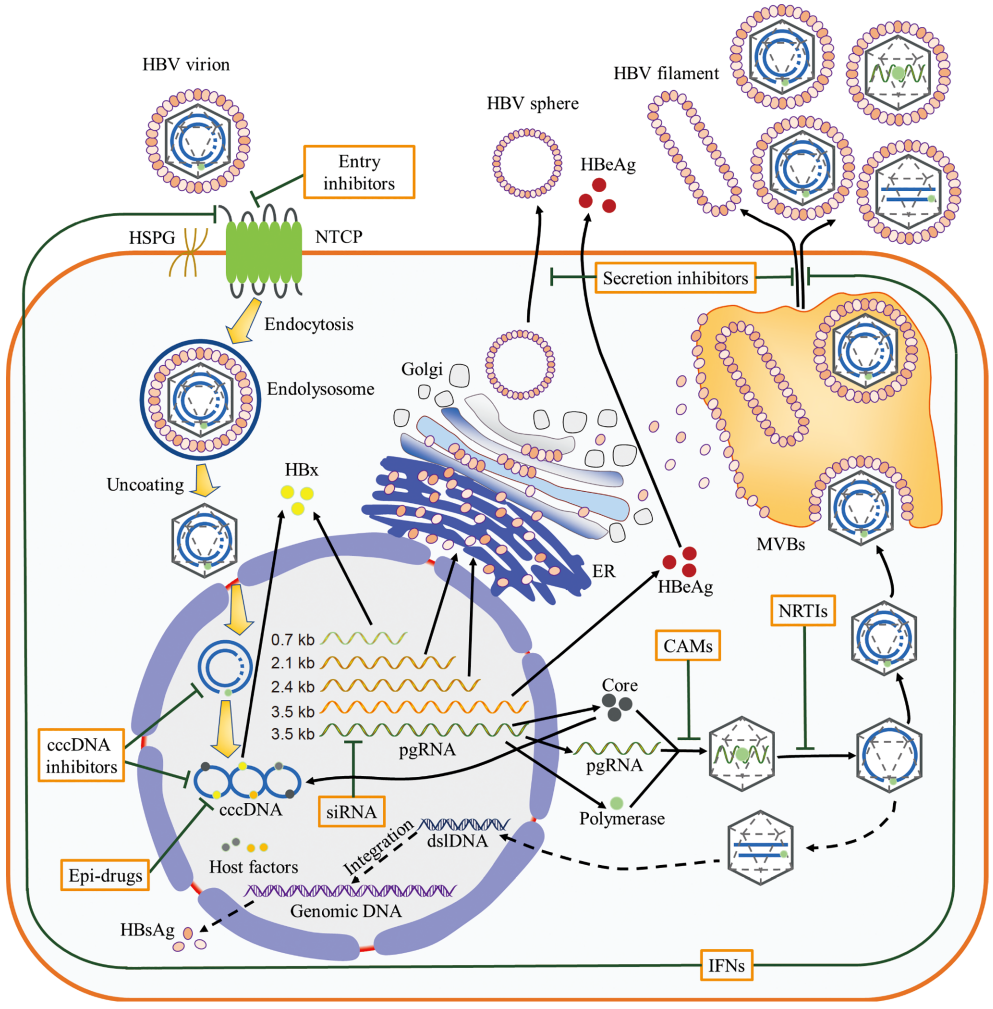


图 1 HBV生活周期和治疗靶标[11]. HBV生活周期包括病毒附着、进入、脱壳、向细胞核的运输、cccDNA形成、转录、翻译、组装、分泌和整合. HBV通过与HSPG的相互作用附着在细胞表面, HBV L蛋白与受体NTCP进一步特异性结合, 介导病毒进入. 进入细胞后, 病毒核衣壳释放并转运至细胞核, 进一步释放出HBV rcDNA. 在细胞核中, rcDNA被转化为cccDNA, 其可作为小染色体在感染细胞的细胞核中持续存在, 并作为病毒mRNA转录的模板. 病毒mRNA被转运到细胞质中, 翻译成病毒的各种蛋白. 与P蛋白一起, pgRNA被包装进核衣壳内并逆转录成子代rcDNA. 成熟的核衣壳或经MVBs途径与HBV表面蛋白进行组装形成成熟的病毒颗粒, 或转运至细胞核将rcDNA转化为cccDNA以建立cccDNA库. 此外, 也会产生一些含有线性双链DNA(double-strand liner DNAdslDNA)的核衣壳, 可释放dslDNA进入细胞核并整合到细胞基因组中或进一步与HBV表面蛋白组装形成有缺陷的病毒粒子释放. 针对HBV生命周期不同步骤的药物或抗病毒剂以红色显示

Figure 1 HBV life cycle and therapeutic targets[11]. The HBV cycle includes virus attachment, entry, uncoating, trafficking to nucleus, cccDNA formation, transcription, translation, encapsulation, secretion, and integration. HBV infection is initiated via interaction with heparan sulfate proteoglycans (HSGP), resulting in a large envelope protein that binds NTCP. After internalization, viral capsids are released and then directed to the nucleus, where the HBV genomes are released. In the nucleus, relaxed circular DNA genomes are converted into cccDNAs, which can persist in the nucleus of infected cells as a minichromosome and serve as a template for viral RNA transcription. Viral mRNAs are transported to the cytoplasm, where they are translated into viral proteins. Together with the viral polymerase, the pgRNA is encapsulated and reverse transcribed within the nucleocapsid into progeny rcDNA. Mature nucleocapsids are then either directed to the MVB pathway for envelopment with HBV envelope proteins or re-directed to the nucleus to establish a cccDNA pool. dslDNA that contains capsid is also produced that can be integrated into the cellular genome or released as a defective virion. Drugs or antiviral agents designed to target different steps of the HBV life cycle are shown in red

quired for transport, ESCRT)进入MVBs并与HBV表面蛋白接触进行囊膜化组装. 最后经MVB或MVB衍生的外泌体与细胞质膜融合而释放HBV病毒粒子[40]. 除了HBV完整病毒颗粒(Dane颗粒)的分泌之外, 还有包括管形颗粒和球形颗粒在内的许多不完整的亚病毒颗粒从感染的肝细胞中释放出来[41]. 其中, 管形颗粒和Dane颗粒具有相同的分泌途径, 而球型颗粒则在内质网腔内进行自组装并通过一般分泌途径释放[42]. 此外, 还有

含HBV RNA和不含病毒核酸的病毒颗粒[43,44].

1 直接抗病毒药物

HBV的生活周期主要分为进入肝细胞、脱壳、rcDNA进入细胞核、cccDNA形成与转录、mRNA出核、核衣壳组装及囊膜化、病毒粒子释放并感染其他肝细胞. 因此, 针对病毒生活周期的不同时期的不同靶标开发相应的药物尤为重要(表1).

1.1 HBV进入抑制剂

如前所述, HBV进入肝细胞首先是由HBsAg与肝细胞膜上HSPGs低亲和性结合, 然后L蛋白与NTCP进行高亲和力结合进入肝细胞(图1). 进入抑制剂主要是通过阻断HBV与肝细胞膜结合发挥抗病毒作用. 肝素、一些小分子化合物和能够结合HSPGs的IFNα诱导因子等都可以抑制HBV进入细胞[13,56,57]. Myrcludex B是一种来源于HBV L蛋白preS1结构域的合成脂肽, 可与HBV preS1竞争性结合肝细胞表面的NTCP从而阻断HBV进入肝细胞. 它是一种不可逆的NTCP受体阻断剂[58], 其对HBV和HDV共感染患者的治疗效果评估已完成II期临床试验[59]. 环孢菌素A类化合物及其衍生物如普萘洛尔、孕酮和氧化固醇等是近期报道的一种肽基脯氨酸基顺/反异构酶和环亲和素[60], 可能干扰NTCP介导的HBV和HDV膜转运[60,61], 目前也在研究开发中. 此外, 氯丙嗪等化学物质能够特异性阻断网格蛋白介导的内吞作用, 进而抑制HBV进入[62]. 抗高血压药伊贝沙坦也能够干扰NTCP的功能并抑制HBsAg表达[63]. EYP001是一种选择性合成FXR激动剂, 可抑制HBV在HepaRG细胞上的感染, 已处在I期临床试验阶段[64].

1.2 cccDNA形成和转录抑制剂

在HBV感染的细胞中, HBV cccDNA可稳定存在且难以清除. 有研究表明, flap-内切核酸酶1(flap-endonuclease 1, FEN1)可以去除rcDNA中的5′-瓣结构, 可能有助于cccDNA的形成[65]. 在HBV初次感染期间, DNA聚合酶κ(polymerase κ, POLK)能够参与完成rcDNA正链的合成[66], DNA连接酶1和3有助于cccDNA的形成[67]. DNA连接酶抑制剂作为一种潜在的抗癌药物正处于开发之中[68], 而此抑制剂也有望成为治疗HBV的潜在药物.

肝脏中富含的多种转录因子和核受体能够与HBV启动子或增强子元件结合以调节HBV转录. PreS1启动子含有肝细胞核因子(hepatocyte nuclear factor, HNF)1(HNF1)和3(HNF3)的结合位点. pre-S2/S启动子的转录由转录因子SP1介导并能响应类视黄醇X受体α(retinoid X receptor α, RXRA)、过氧化物酶体增殖物激活受体α(peroxisome proliferator-activated receptor α, PPARα)和HNF4A. 增强子I含有HNF1, HNF3和CCAAT-增强子结合蛋白(CCAAT-enhancer-binding protein, CEBP)的结合位点. 此外, pre-C/C启动子和两种增强子都含有核受体的结合位点, 包括HNF4A, RXRA, PPARα, 核受体亚家族2组F成员(nuclear receptor subfamily 2 group F member, NR2F2或COUPTF)1和2以及核受体亚家族2组C成员1(NR2C1或TR2). 以上这些转录因子的靶向抑制化合物均可以抑制HBV复制[11,25,69]. 此外, HBV cccDNA小染色体的DNA甲基化和组蛋白修饰的表观遗传修饰能够影响HBV cccDNA的转录[70]; 细胞因子也能够与cccDNA相互作用参与表观遗传修饰, 从而促进或抑制HBV cccDNA转录[71~73]; HBx可以将损伤特异性DNA结合蛋白E3泛素连接酶重新定位至染色体结构维持蛋白(structural maintenance of chromosomes, SMC)5和6以进行降解, 从而减轻对HBV cccDNA转录的抑制作用[74]; HBx也可以抵消SETDB1蛋白介导的H3组蛋白的二、三甲基化(H3K9me3)和HP1招募以激活cccDNA转录[71]. 因此, HBx或将成为一种有吸引力的病毒治疗靶点. 目前调节表观遗传修饰的药物也已经用于开发治疗癌症或病毒感染的患者[75,76]. GS-5801是赖氨酸脱甲基酶-5的抑制剂, 作为一种肝靶向前药正在进行I期临床试验, 它也是首批在HBV感染患者中进行试验的调节表观遗传修饰的药物之一[77]. 这些药物或许能够用于灭活肝细胞中的cccDNA. 此外, 还有学者从小分子药物类似成分的小分子文库中筛选出CCC-0975和CCC-0346两种结构相关的双取代磺胺类药物. 这两种小分子虽然不能抑制病毒聚合酶的活性和直接抑制HBV DNA的复制, 但可以通过抑制rcDNA去蛋白化进而阻断rcDNA向cccDNA的转化[78,79]. 小分子化合物C646则可以通过特异性抑制p300和CBP组蛋白乙酰转移酶来抑制HBV[80].

1.3 基因编辑

基因编辑也是常用的药物开发工具之一. 锌指核酸酶(zinc-finger nucleases, ZFNs)、转录激活剂样效应物(transcription activator-like effector nucleases, TA-

表 1　用于治疗HBV感染的直接作用抗病毒药物和宿主靶向药物

Table 1　Direct-acting antivirals and agents that target host factors in development for treatment of HBV infection

药物	作用机制	临床研究阶段	开发商	临床试验注册号
Myrcludex B	Entry	Phase 2	Hepatera, Russia	NCT02888106 NCT02637999
ARC-520	siRNA	Terminated	Arrowhead	NCT02452528 NCT02604212 NCT02604199 NCT02738008 NCT02065336 NCT02577029
ARC-521	siRNA	Terminated	Arrowhead	NCT02797522
ARO-HBV	siRNA	Phase 1/2	Arrowhead	NCT03365947
ARB-1467 (TKM-HBV)	siRNA	Phase 2	Arbutus Biopharma, USA	NCT02631096
ARB-1740	siRNA	Terminated	Arbutus Biopharma, USA	Sponsor's web site
AB729	siRNA	Phase 1	Arbutus Biopharma, USA	Sponsor's web site
LUNAR^{TM}-HBV	siRNA	Preclinical	Arcturus	Sponsor's web site
DCR-HBVS	siRNA	Preclinical	Dicerna, USA	Sponsor's web site
ALN-HBV	siRNA	Phase 1/2	Alnylam	NCT02826018
Vir-2218 (ALN-HBV02)	siRNA	Phase 1/2	Alnylam	NCT03672188
BB-103(Hepbarna, BB-HB331)	siRNA	Preclinical	Benitec, Australia	Sponsor'S web site
IONIS-HBVLRx (GSK3389404)	Antisense oligonucleotide	Phase 2	Ionis Pharmaceuticals, with GlaxoSmithK-line, UK	NCT03020745
IONIS-HBVRx (GSK3228836)	Antisense oligonucleotide	Phase 2	Ionis Pharmaceuticals, with GlaxoSmithK-line, UK	NCT02981602
RG6004 (RO7062931)	Locked Nucleic Acid	Phase 1/2	Roche, Switzerland	NCT03038113[45]
GS-5801	Epidrug	Phase 1	Gilead	Sponsor's web site
AB-452	RNA destabilizer	Preclinical	Arbutus Biopharma, USA	Sponsor's web site
EBT106	CRISPR	Preclinical	Excision BioTherapeutics	Sponsor's web site
HBV	CRISPR	Preclinical	Intellia Therapeutics	Sponsor's web site
HBV	CRISPR	Preclinical	Precision Biopharma	Sponsor's web site
JNJ-379 (JNJ-56136379)	Capsid	Phase 2	Janssen Sciences, Belgium	NCT03361956
JNJ-440 (JNJ-64530440)	Capsid	Phase 1	Janssen Sciences, Belgium	NCT03439488
Morphothiadin (GLS4)	Capsid	Phase2	HEC Pham, China	NCT03638076[46]
GLP-26	Capsid	Preclinical	Emory University, USA	[47]
EP-027367	Capsid	Preclinical	Enanta Pharmaceuticals, USA	[48]
NVR 3-778 (AL-3778)	Capsid	Phase 1/2	Novira	NCT02112799NC-T02401737[49]
Bay 41-4109	Capsid	Phase 1	AiCuris, Germany	Sponsor's web site[50]
AB-423	Capsid	Phase 1	ArbutusBiopharma, USA	Sponsor's web site
AB-506	Capsid	Preclinical	Arbutus Biopharma, USA	Sponsor's web site
QL-007	Capsid	Phase 1	Qilu Pharmaceutical, China	NCT03244085

(续表1)

药物	作用机制	临床研究阶段	开发商	临床试验注册号
QL-0A6a	Capsid	Preclinical	Qilu Pharmaceutical, China	Sponsor'S web site
RO7049389	Capsid	Phase 2	Roche, Switzerland	NCT03717064 NCT03570658
RO6864018	Capsid	Phase 2	Roche, Switzerland	NCT02391805
RG-7907	Capsid	Phase 1	Roche, Switzerland	NCT02952924
ABI-H0731	Capsid	Phase 1b/2a	AssemblyBiosciences, USA	NCT03109730
ABI-H2158	Capsid	Phase 1	Assembly Biosciences, USA	NCT03714152
CB-HBV-001	Capsid	Preclinical	Zhimeng Biopharma, China	Sponsor'S web site[51]
Besifovir	Polymerase	Phase 3, Approved in South Korea	Il Dong Pharmaceutical, South Korea	NCT01937806
DA-2802(tenofovir disoproxil orotate)	Polymerase	Phase 3	Dong-A ST Co., Ltd., South Korea	NCT02967939
Metacavir	Polymerase	Phase 2	Guangzhou Yipinhong Pharmaceutical, China	NCT02965859
CMX157 (tenofovir Exalidex)	Polymerase	Phase 2	ContraVir Pharmaceuticals	NCT02710604
HS-10234	Polymerase	Phase 3	Jiangsu Hansoh Pharmaceutical, China	Sponsor's web site
CKD-390 (Tenofovir disoproxil aspartate)	Polymerase	Phase 3	Chong Kun Dang, South Korea	NCT02805738
RNase H inhibitors	RNaseH	Preclinical	Arbutus	Sponsor's web site
REP 2055 (REP 9AC)	HBsAg	Phase 2	Replicor, Canada	NCT02646163[52]
REP 2139	HBsAg	Phase 2	Replicor, Canada	NCT02565719[53,54]
REP 2165	HBsAg	Phase 2	Replicor, Canada	NCT02565719[54]
RO7020322 (RG7834)	HBsAg	Terminated	Roche, Switzerland	NCT02604355[55]

LENs)和成簇的规律间隔短回文序列(clustered regularly interspaced short palindromic repeats, CRISPR)及其相关蛋白内切酶(CRISPR associated nuclease, Cas)是目前已经证实可以用来抑制HBV复制的3种内切酶[81]. HBV特异的ZFN能够有效裂解细胞中HBV DNA, 进而抑制其复制[82]. TALENs可以利用特定的DNA结合结构域(customizable DNA-binding domains)识别HBV的序列以改变HBV基因组序列, 从而减少HBV蛋白的产生、降低pgRNA和cccDNA的水平[83]. CRISPR/Cas9利用HBV特异的gRNA(guide RNA)切割HBV DNA的两条链, 导致双链DNA断裂修复, 以此失活甚至消除cccDNA及整合在宿主基因组中的HBV DNA片段. 有研究表明, 利用CRISPR/Cas9系统可以将细胞中HBV的病毒载量和cccDNA水平分别减少0.1%和10%[84]. 以上这些研究证明了基因编辑技术具有很大的潜力, 被开发用于治疗甚至治愈慢性HBV感染患者. Excision BioTherapeutics和Intellia Therapeutics公司分别公布了他们第一个用于HBV感染的CRISPR/Cas9相关候选治疗方案. 然而, CRISPR/Cas9系统对患者的脱靶效应是一个值得关注的问题[85], 在基因编辑过程中诱导的p53介导的DNA损伤反应也可能妨碍其临床应用[86].

1.4 RNA干扰

RNA干扰(RNA interference, RNAi)技术可用于抑制病毒基因表达, 有望被开发用于抗病毒治疗. 其原理是首先设计小干扰RNA(small interfering RNA, siRNA)以靶向特定的病毒mRNA序列. 然后再将它们递送到肝细胞中, siRNA与病毒mRNA互补杂交, 进而导致双链RNA的降解[87]. 第一种针对HBV开发的siRNA是ARC-520[88], 它能够有效降低HBV感染的黑猩猩和患者中HBsAg, HBeAg, HBcAg和HBV DNA的滴度[89]. 但在HBeAg阴性的黑猩猩和患者中观察到的HBsAg减少明显低于HBeAg阳性组, 这可能是因为ARC-520靶向以cccDNA而不是整合于宿主基因组中的HBV DNA

为模板转录出的转录本. 这也催生了第二代siRNA, 即ARC-521[90]. ARC-520/521需要使用复杂的递送制剂, 该制剂为动态多聚物(dynamic poly-conjugates, DPC), 是Arrowhead公司开发的用于将siRNA靶向递送至肝细胞的递送平台. 虽然ARC-520/521在临床前模型及临床试验中表现出了概念验证阶段的有效性, 但由于其临床前期研究中因DPC平台表现出的肾脏毒副作用而终止. 为实现肝脏特异性靶向并使肾脏暴露最小化, Alnylam 公司开发的ALN-HBV将HBV特异性siRNA缀合至3个半乳糖胺(GalNAc)簇. 该策略可实现ALN-HBV与表达在肝细胞表面的唾液酸糖蛋白(asialoglycoprotein receptor, ASGPR)结合而特异性地进入肝细胞. 该公司随后又采用增强稳定化学-Elus(ESC+)GalNAc共轭技术开发了用于慢性乙肝治疗的 RNAi 新药ALN-HBV02(Vir-2218), 该药旨在抑制所有HBV蛋白的表达, 目前正处于I/II期临床试验阶段[91]. Arrowhead公司的研究人员使用其专有的皮下给药运载工具开发了ARO-HBV. 初步结果显示, ARO-HBV可长期显著降低HBV DNA和病毒抗原HBsAg; 在整合HBV DNA而衍生出的HBsAg模型中同样也表现出了对HBsAg的抑制作用, 且对大鼠有比较低的毒性. ARO-HBV目前正在进行I/II期临床试验[92], 有望与其他抗病毒化合物协同作用. 此外, 采用类似的方法开发的还有反义寡核苷酸(GSK3389404和GSK3228836), 锁定核酸(RG6004)和RNA去稳定剂(AB-452)等[11,91]. 针对HBV的siRNA目前仍处于试验阶段, 需要更多的研究来解决药物疗效和安全性问题.

1.5 核衣壳抑制剂

HBV核心蛋白(HBV core protein, HBc)具有多种功能, 它不仅可以自组装形成核衣壳, 影响pgRNA的衣壳化, 还可以在细胞核内招募组蛋白甲基化酶调节cccDNA及宿主相关基因的表达. 病毒核衣壳的形成和pgRNA包装是病毒生活周期的关键步骤, 因此, HBc有可能成为抗病毒药物的靶点. 目前针对这一靶点已经开发出两类主要的核衣壳组装调节剂(capsid assembly modulators, CAMs): (1) 苯丙烯酰胺类和氨磺酰苯甲酰胺类化合物(phenylpropenamides, PPAs)可以干扰pgRNA的包装并加速未成熟空核衣壳样颗粒的形成; (2) 杂芳基二氢嘧啶类化合物(heteroaryldihydropyrimidines, HAPs)可以诱导聚合和异常的核衣壳结构形成及破坏完整的核衣壳. 目前正在进行研究或临床试验的CAMs有AT-61, AT-130, DVR-01, DVR-23, NVR3-778, HAP-1, HAP-18, NVR-010-001-E2, GLS4, Bay 41-4109, BAY 38-7690, BAY 39-5493, RO7049389, RO6864018, JNJ-379, JNJ-440, ABI-H0731, ABI-H2158, AB-506, AB-423, BCM-599和QL-007等[11,79]. AB-506和ABI-H2158正处于临床前研究阶段; JNJ-379是一种具有双重功能的CAM, 既可以干扰cccDNA的从头合成, 又可以阻断病毒核衣壳的组装. 相关研究表明, JNJ-379在给药的28 d中出现良好的耐受, 并且优化的剂量具有有效的抗病毒活性[93]; ABI-H0731在I期临床试验中安全且耐受方面表现良好并具有有效的抗病毒活性[94]; RO7049389在慢性HBV感染患者中具有强大的抗病毒活性, 安全及耐受性方面也表现良好[95]. 此外, 研究人员还发现了一类新的HBV核衣壳抑制剂CB-HBV-001, 它是具有全新骨架的吡唑类化合物. CB-HBV-001以剂量依赖的方式抑制含有HBV pgRNA和DNA的核衣壳形成, 对不同基因型HBV和对核苷类似物(nucleos(t)ide reverse transcriptase inhibitors, NRTIs, 也被称为 nucleos(t)ide analogs, NAs)治疗产生耐受的HBV均有抑制作用[51]. Bay 41-4109是目前研究最详细的一种HAP, 具有双重的作用机制, 在低浓度时可引起核衣壳的不适当组装, 高浓度时则会促进核衣壳的错误组装及降低核衣壳的稳定性[96,97].

1.6 HBV聚合酶抑制剂

HBV聚合酶(polymerase, P)是一个多功能蛋白, 含有末端蛋白(terminal protein, TP)区、间隔区(space)、逆转录酶(reverse transcriptase, RT)活性区、核糖核酸酶H(ribonuclease, RNaseH)区. NRTIs可以靶向P蛋白的RT区, 干扰pgRNA逆转录合成病毒DNA, 是慢性HBV感染治疗的中坚力量. 第二代NRTIs药物如恩替卡韦、替诺福韦和克拉夫定可以抑制P蛋白介导的逆转录起始[98], 从而有效抑制HBV复制, 但对具有较长半衰期的cccDNA水平和活性几乎没有影响, 因此并不能治愈HBV感染[6]. 所以, 患者需要终身服药, 同时也存在一定耐药性和药物毒性等方面的问题[99]. 目前正在开发的多种NRTIs药物旨在对现有药物进行改良, 包括Besifovir(LB80380)[89], Metacavir[100], Tenofovir的两种前药CMX157和DA-2802. 这些药物具有改善的药理学性质并且可以减少一些副作用[101], 但是否比现有药物具有实质性的优势还需要进一步研究. HS-10234是新一代单磷酰胺单酯类的替诺福韦前药, 在血浆中非常稳定,

目前正在进行Ⅲ期临床试验. RNase H是HBV P蛋白具有的特异性酶活性, 也是P蛋白发挥功能所必需的, 因此, RNase H有望成为一个好的HBV治疗靶点. 近年来已经鉴定了多种RNase H抑制剂, 包括β-噻嗪素和羟化托酚酮[102,103], 这些抗HBV抑制剂正在开发中, 有望与现有治疗手段相结合以达到更好的抑制病毒复制的效果.

1.7 HBV分泌抑制剂

在HBV感染患者中常伴有HBsAg的高表达. HBsAg能够影响机体免疫系统反应, 从而导致持续性慢性感染, 因此, 控制HBsAg的分泌有助于机体免疫功能的恢复. REP9AC能够有效抑制HBV感染患者肝细胞释放HBsAg, 该药目前正处于II期临床试验阶段[52]; REP2139是一种核酸聚合物(nucleic acid polymer, NAP), 这类药物的具体作用机制还不是很清楚, 可能通过干扰亚病毒粒子组装抑制HBsAg的合成释放[53]. 其他抑制HBV颗粒的组装和释放的葡萄糖苷酶抑制剂和核酸聚合物也在开发中[53,54,104,105].

2 免疫疗法

免疫细胞和细胞因子可以介导HBV感染的细胞裂解和非细胞裂解清除[106]. 每年约有0.5%~1%的患者在慢性感染数十年后通过其免疫系统激活来抑制HBV复制、清除HBsAg并出现血清学转换[107]. 因此, 研发相关药物以激活细胞因子介导的抗病毒先天免疫反应, 并进一步恢复机体适应性免疫是一种治疗HBV感染行之有效的方法[11]. 免疫疗法的直接目标是恢复患者对HBV感染的免疫力, 在不进行抗病毒治疗的情况下完全并长期控制病毒, 并显著增加功能性治愈患者的比例(表2).

2.1 先天免疫系统刺激

先天免疫反应不是抗原特异性的, 但会产生抗病毒和T细胞极化相关的细胞因子, 并改变肝脏微环境以促进免疫反应. HBV复制虽然不会诱导肝细胞产生细胞因子, 但病毒对靶向其生活周期不同步骤的细胞因子敏感[115]. 模式识别受体(pattern recognition receptor, PRR)或细胞质核酸传感器的激活导致干扰素(interferon, IFN)或炎性细胞因子如肿瘤坏死因子(tumor necrosis factor, TNF), 白介素(interleukin, IL)1, IL6, IL12和IL18的分泌[116]. toll样受体(toll-like receptor, TLR)信号传导控制专职抗原递呈细胞(antigen presenting cell, APC)中吞噬体的成熟, 以确保APC中主要组织相容性复合物II(major histocompatibility complex II, MHCII)呈递病原体衍生的抗原[117]. 此外, 刺激非实质肝细胞如kupffer细胞和单核细胞, 可以改变肝脏环境, 从而促进抗病毒反应[118]. 因此, 激活先天免疫应答可导致具有抗病毒活性的细胞因子的产生, 促进抗原递呈和改变肝脏微环境. 然而, 细胞因子如IFNα, IFNβ和TNF也可以诱导慢性感染患者的免疫耐受, 这可能会限制持续的免疫控制[119,120].

2.1.1 干扰素

IFNα是首个用于HBV感染治疗的药物, 它可以诱导细胞内具有抗病毒活性的IFN刺激基因(IFN-stimulated genes, ISGs)的表达, 从而抑制HBV mRNA的翻译和病毒蛋白的合成, 进而抑制病毒的复制[121,122]. 在小鼠中, 抗病毒作用主要由I型IFN介导, 其控制HBV转录、病毒RNA和核衣壳的稳定性; I型IFN、III型IFN、淋巴毒素β和TNF可导致HBV cccDNA从感染细胞核中丢失[123,124]. 淋巴毒素受体通路在慢性HBV感染患者的肝脏中具有活性[125], 高剂量的IFNα和淋巴毒素β受体(lymphotoxin β receptor, LTβR)激动剂可以通过诱导核脱氨酶载脂蛋白B mRNA编辑酶催化亚基(apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like, APOBEC)3A或3B进而诱导HBV感染的人原代肝细胞或HepaRG细胞中cccDNA的非细胞溶解降解[124]. IFNα可以上调患者肝脏中APOBEC3A水平[126], LTβR激动剂上调APOBEC3B[125]. 在Cre介导的重组HBV cccDNA的细胞系中过表达APOBEC3A能够导致cccDNA的减少[127]. 也有研究表明, IFNα还可以抑制包被HBV pgRNA的核衣壳形成, 促进HBV cccDNA的降解, 参与cccDNA转录的表观遗传修饰[128]. 然而, IFNα导致的副作用限制了其应用.

2.1.2 TLR激动剂

TLR是机体免疫系统中重要的病原体识别受体, 可以感知外源病原体的存在, 进而激活炎性细胞因子的释放和适应性免疫应答[79]. 目前在动物模型中发现的具有抗病毒作用的TLRs除了TLR3外, 其他TLRs都需要信号适应蛋白MyD88. TLRs在骨髓细胞(树突状细胞、单核细胞、巨噬细胞)上呈高表达, 在人肝细胞上除TLR1, TLR2, TLR3和TLR4外呈低表达. 有研究表明, HBV可以通过下调TLR以逃避机体天然免疫应答, 而TLR与特定配体结合可以通过激活机体天然免疫和

表 2　用于治疗HBV感染的免疫调节药物

Table 2　Immunomodulatory therapy for treatment of HBV Infection

药物	作用机制	临床研究阶段	开发商	临床试验注册号
EYP001	FXR agonist	Phase 1	Enyo Pharma, France	NCT03469583
CRV431	Cyclophilin inhibitor	Phase 1/2	ContraVir Pharmaceutical	Sponsor's web site
NVP 018 (BC556)	Cyclophilin inhibitor, IRF-9	Preclinical	NeuroVivePharmaceutial	Sponsor's web site
APG-1387	Apoptosis	Phase 1	Asscentage Pharma, China	Sponsor's web site[108]
Birinapant	Apoptosis	Terminated	TetraLogic Pharmaceutical	NCT02288208
NJK14047	p38 MAPK inhibitor	Preclinical	Kyung Hee University, Korea	[109]
GS-9620	TLR7 agonist	Phase 2	Gilead, USA	NCT02579382
RO6864018 (RG7795 and ANA773)	TLR7 agonist	Phase 2	Roche, Switzerland	NCT02391805
RO7020531	TLR7 agonist	Phase 1	Roche, Switzerland	NCT02956850
RG7854	TLR7 agonist	Phase 1	Roche, Switzerland	NCT02956850
AL-034	TLR7 agonist	Phase 1	Janssen Sciences, Belgium	NCT03285620
GS-9688	TLR8 agonist	Phase 2	Gilead, USA	NCT03491553
AIC649	Immune modulator	Phase 1	AiCuris, Germany	[110]
Thymalfasin	Immune modulator	Phase 4	Huashan Hospital, China	NCT03448744
GC-1102	Immune modulator	Phase 2	Green Cross	NCT03519113[111]
Inarigivir (GS-999 and SB 9200)	RIGI and NOD2 agonist	Phase 2	Spring Bank	NCT02751996 NCT03434353
Nivolumab	PD-1 blockade	Phase 1/2	BMS	NCT01658878[112]
CBT 501	PD-1 blockade	Phase 1/2	CBT Pharmaceuticals, Australia	NCT03655613
INO-1800	Therapeutic vaccine	Phase 1	Inovio Pharmaceuticals	NCT02431312
TG1050	Therapeutic vaccine	Phase 1	Transgene, France	NCT02428400
GS-4774	Therapeutic vaccine	Terminated	Gilead, USA	NCT02258581
ABX-203	Therapeutic vaccine	Phase 4	Abivax, France	NCT02249988
DV-601	Therapeutic vaccine	Phase 1	Dynavax, Poland	NCT01023230
HB-110	Therapeutic vaccine	Phase 1	Genexine, South Korea	NCT01641536 NCT00513968[113]
HepTcell (FP-02.2)	Therapeutic vaccine	Phase 1	Altimmune	NCT02496897
JNJ-64300535	Therapeutic vaccine	Phase 1	Janssen Sciences, Belgium	NCT03463369
VR-CHB01	Therapeutic vaccine	Preclinical	Vical, USA	Sponsor's web site
VBI-2601	Therapeutic vaccine	Preclinical	VBI Vaccines, USA with Brii Biosciences	Sponsor's web site
TomegaVax HBV	Therapeutic vaccine	Preclinical	TomegaVax	Sponsor's web site
MVA-VLP-HBV	Therapeutic vaccine	Preclinical	GeoVax and CaroGen	Sponsor's web site
HBV vaccine+IFN+α2b+IL-2	Therapeutic vaccine	Phase 2/3	Tongji Hospital	NCT02360592
CVI-HBV-002	Therapeutic vaccine	Phase 1/2	Cha Vaccine Institute	NCT02693652; KCT0001249
LTCR-H2-1	T cell receptor	Preclinical	Lion TCR, Singapore	Sponsor's web site
IMM Tav	T cell immune regulation	Preclinical	Immunocore, USA	[114]

适应性免疫促进干扰素的分泌、T淋巴细胞共刺激和B淋巴细胞分化等来抑制HBV的复制[129]. 激活肝细胞上的TLR1，TLR2，TLR3和TLR4对HBV具有直接的抗病毒作用[130]. 在HBV转基因小鼠中，注射TLR3，TLR4，

TLR5, TLR7或TLR9的激活剂可以降低HBV的复制[131]; 在土拨鼠中, TLR7和TLR9的激活可以抑制土拨鼠肝炎病毒(woodchuck hepatitis virus, WHV)的复制[132]. 此外, TLR诱导的炎性单核细胞中产生的TNF可以增加小鼠淋巴细胞聚集以支持肝内T细胞的活化增殖[133]; 不同的TLRs可以诱导刺激不同细胞因子产生的转录因子的激活, 如NF-κB和干扰素调节因子3(interferon regulatory factor 3, IRF3)和IRF7[123].

GS-9620是一种TLR7口服激动剂, 在HBV感染的黑猩猩中, 可以显著降低黑猩猩血清中HBV DNA和HBsAg以及肝中HBV DNA的水平[134]; 但在经抗病毒药物治疗而病毒复制被抑制的慢性乙型肝炎患者中, GS-9620并不能有效降低患者血清中的HBsAg水平[135]. 其他TLR7激动剂如RO6864018正处于II期临床试验阶段, RG7854和AL-034正处于I期临床试验阶段[136]. TLR8激动剂被认为是先天免疫的重要激活因子, 因为它们可以诱导人肝脏中单核细胞分泌IL-12和IL-18, 从而导致肝内固有免疫细胞如黏膜相关T细胞和NK细胞(natural killer cell)产生IFN-γ[137]. 在用IL-12或IL-18处理的情况下, IFN-γ在黑猩猩中可以介导HBV的清除, 并且可以抑制HBV转基因小鼠、土拨鼠和CHB患者中的HBV复制[138,139]. 在体外实验中, IL-12可以挽救耗尽的HBV特异性$CD8^+$ T细胞的抗病毒功能[140]. GS-9688是一种口服TLR8激动剂, 可以在刺激单核细胞后在肝内检测到IFN-γ产生, 目前正处于II期临床试验阶段[141]. CpG寡脱氧核苷酸是TLR9激动剂, 其处理浆细胞样树突状细胞(plasmacytoid dendritic cell, pDC)和B细胞后可以诱导IFN-α的产生[142]. 用CpG 21798与恩替卡韦(entecavir, ETV)联合治疗土拨鼠可以比ETV单药治疗更早地将WHV DNA, 特别是WHV表面抗原(WHV surface antigen, WHsAg)降低到检测限以下, 并延迟病毒复发[143]. AIC649是一种失活的副痘病毒颗粒制剂, 可通过激活骨髓细胞中的补体和TLR依赖性(TLR9)和非依赖性信号传导, 促进辅助T细胞介导的反应和IFN-α等细胞因子的释放[144], 在土拨鼠模型中可以降低HBsAg的水平[110], 在慢性HBV感染患者的I期临床试验中安全性也表现良好. 当TLR9激动剂与核苷酸类似物联合用药时, 可以表现出累加效应. Riboxxol是一种合成的50 bp的双链RNA, 可激活TLR3[145]. Riboxxol在培养的人原代肝细胞中可以控制HBV的复制并降低cccDNA水平, 具有长效抗病毒作用[129]. 一系列TLR激活剂的研发正在进行中, 而关于这些潜在药物的功效和毒性必须仔细进行评估, 以提高其治疗指数.

2.1.3 细胞质核酸传感器刺激因子

细胞质中的RNA传感器包括RNA解旋酶DExD/H-box解旋酶58(DDX58/RIG-I)和IFIH1/MDA5(interferon induced with helicase C domain 1), 可以通过激活IRF3和NF-κB诱导细胞因子的产生[146]. MDA5识别细胞中的双链RNA, 而RIG-I识别5′三磷酸化的病毒RNA. RIG-I不仅可以激活炎性小体并诱导细胞凋亡, RIG-I自身的激活也可以诱导针对HBV的抗病毒反应[147]. 小分子核酸杂交体SB-9200是二核苷酸SB-9000的前体药物, 可以激活RIG-I和核苷酸结合寡聚化结构域2(nucleotide binding oligomerization domain containing 2, NOD2), 进而刺激IFN产生并对HBV聚合酶具有直接抗病毒作用. SB-9200可以激活土拨鼠肝脏中的RIG-I表达[148], 但对WHV DNA和WHsAg的水平并没有很好的剂量依赖抑制效应, 并且在停药后会出现病毒复制的反弹[149]. 目前, 在SB-9200治疗后接替以核苷类似物治疗的方案正在进行II期临床试验.

环鸟苷单磷酸-腺苷酸单磷酸合成酶(cyclic guanosine monophosphate-adenosine monophosphate synthase, cGAS)可以识别细胞浆液中的双链DNA, 将其加工形成环二核苷酸(cyclic dinucleotides, cGAMP), 并被跨膜蛋白173(transmembrane protein 173, TMEM173 or STING)进一步识别, 从而激活先天性免疫应答. 小鼠STING激动剂DMXAA在巨噬细胞中可以诱导IFN-α/β介导的细胞因子释放, 从而抑制小鼠肝细胞中的HBV复制[150]. 关于STING激动剂的研究还处在临床前的评估阶段[151].

2.2 激活适应性免疫

适应性免疫应答的激活对于控制和治愈HBV感染是关键的, 并且多种信号通路可能有助于其激活[10]. 目前开发的恢复针对肿瘤抗原特异性免疫应答的药物主要包括靶向受感染细胞表面分子的抗体、重新激活抗原特异性T细胞的检查点抑制剂、使用基因修饰T细胞的细胞疗法和治疗性疫苗[152,153], 这些药物对HBV感染均可能表现出抗病毒效果.

2.2.1 免疫检查点抑制剂

机体的免疫系统都依赖于免疫检查点分子. 在慢性乙肝患者中, HBV特异性T细胞是HBV感染免疫控制所必需的, 其抗病毒功能主要是介导急性HBV感染的肝细胞的裂解[123]. HBV特异性T细胞的耗竭导致机

体无法对病毒进行控制, 而T细胞的耗竭与共抑制性受体PD1, CTLA-4, TIM-3, T细胞免疫球蛋白, CD244和BTLA的过度表达有关[154,155]. 通过校正这些共抑制性受体和共刺激信号(41BB和IL-12)之间的平衡可以恢复T细胞的功能, 增强机体免疫力[156]. 有研究表明, 在HBV感染相关的肝癌患者中, PD-1和TIM-3均具有不同程度的表达水平, 并且与肝炎和T细胞衰竭程度密切相关[157]. PD-1是诱导$CD8^+$T细胞衰竭的主要抑制性受体, 使用抗PD-L1的抗体阻断PD-1与PD-L1的相互作用可导致肝脏内HBV特异性T细胞应答增加[158,159]. 给慢性HBV或HCV感染的肝癌患者使用PD-1抑制剂Nivolumab后, 不会引起严重的肝毒性[112], 但是其治疗效果仍需要进一步研究. BTLA信号通路被阻断后, HBV抗原对免疫细胞的抑制性明显降低[155]. 免疫检查点抑制剂有可能成为治疗慢性HBV感染的有效疗法, 但其疗效和安全性还需要进一步的评估[9].

2.2.2 T细胞重建

T细胞重建旨在通过基因工程T细胞以靶向和消除HBV感染的肝细胞[2]. 通过将具有HBV免疫力的供体骨髓移植到慢性感染患者体内的免疫重建, 虽然可以清除肝脏中的HBV[152], 但是同种异体干细胞移植的严重副作用(如免疫排斥和高死亡率)限制了其应用. 使用经遗传工程改造以表达HBV特异性受体的自体T细胞以重建免疫可用于治疗慢性HBV感染, 预防HBV相关并发症或治疗HBV相关HCC[160]. 嵌合抗原受体(chimeric antigen receptor, CAR)的结构包括胞外靶标结合结构域、胞外铰链区、跨膜的嵌合受体-锚定区域和一个或多个细胞内信号传导结构域. 靶标结合结构域衍生自免疫球蛋白单链可变片段的轻链和重链序列[161], 可用于治疗持续性病毒感染[162]. CAR修饰的T细胞与HBV感染的肝细胞的结合可以触发增殖或激活信号以启动有效的抗HBV T细胞应答[163], 这种方法已经在HBV动物模型中进行了一定的验证, 如针对肝细胞表面的HBsAg的CAR-T细胞可以安全有效地消除小鼠中HBV感染的细胞[164], 而将T细胞受体(T cell receptor, TCR)工程改造的T细胞给予患者的试验研究也证实了该治疗方案的安全性[160]. 此外, 在人源化肝脏的小鼠中观察到了表达CAR或工程化TCR的T细胞的细胞裂解和非细胞裂解性抗病毒作用[165]. 因此, 该方法对于治疗HBV相关HCC或慢性HBV感染具有一定的潜力.

2.2.3 治疗性疫苗

慢性乙型肝炎中治疗性疫苗接种的目标是诱导足够的抗HBV免疫应答以消除或治愈HBV感染的肝细胞而不会对宿主细胞造成过度损伤, 防止病毒扩散到新的肝细胞, 并达到长期的病毒控制. 目前研究的治疗性疫苗大多关注于常规表面抗原疫苗[166,167]、表面抗原与抗HBsAg的免疫复合物、T细胞疫苗[168]和DNA疫苗[169,170], 并且已经取得了实质性进展.

GS-4774是一种完整的热灭活酵母疫苗, 可表达HBV抗原(HBV X, HBsAg和HBcAg)并诱导$CD4^+$和$CD8^+$T细胞应答[171]. TG-1050是一种重组非复制型人腺病毒, 可表达多种基因D型HBV抗原(HBcAg和P蛋白), 通过刺激T淋巴细胞介导的免疫反应直接杀死患者体内HBV感染的细胞或抑制HBV复制[172]. ABX203是由HBsAg和HBcAg重组而成, 对HBsAg和HBcAg具有强有力的免疫效应[173]. INO-1800是一种编码HBsAg和HBcAg的多抗原DNA疫苗, 在经恩替卡韦或替诺福韦治疗的患者中单独使用或与编码IL-12的INO-9112联合使用[174]. DV-601是将HBsAg和HBcAg与ISCOMATRIX®佐剂结合使用, 临床试验结果表明其有很好的安全性和免疫原性[175]. HepTcell(FP-02.2)是一种由9种合成肽组成的疫苗, 这些肽来源于HBV最保守的结构域, 含有高密度的$CD4^+$和$CD8^+$ T细胞表位, 受多个人类白细胞抗原(human leukocyte antigen, HLA)等位基因限制, 适用于多种HBV基因型[176]. 此外还有含有乙型肝炎免疫球蛋白和粒细胞-巨噬细胞集落刺激因子的HBV疫苗, 以及含有IFN-α2b和IL-2的HBV疫苗等.

3 小分子HBV抑制剂的高通量筛选

随着高通量技术的发展和小分子化学库的不断扩大, 研究人员开始进行大规模的筛选以鉴定可以抑制HBV的化合物. 抗病毒化合物的筛选首先需要开发具有感染性或复制性细胞培养系统的小型平台以及高通量病毒检测系统, 且需要整个系统的灵敏度高、结果稳定可重复, 此外还需要进一步开发潜在的化合物库[177,178]. 使用这种系统的构建模块已经建立了HBV感染性或复制性细胞培养系统[179], 开发并进行了多种HBV抑制剂的筛选鉴定[180].

研究表明, 一些临床中针对其他疾病的药物能够作用于宿主多种不同的通路, 因此可以比较药物的作用机制重新调整或定位药物用途[181]. 通过运用高通量筛选方法可以从临床中用于其他疾病治疗的药物或者现有化合物库中筛选出能够抑制HBV复制或感染的药物作为新的抗HBV感染的候选药物[182], 以期可用于抗

病毒感染的治疗[183,184]. 这不仅可以有效缩短药物开发的时间, 还能够节约药物研发的成本.

4 未来发展方向

尽管长期的抗病毒治疗能够使和病毒性肝炎相关的肝纤维化、肝硬化以及肝癌患者的死亡率降低, 但是目前慢性乙肝仍很难完全治愈, 主要障碍是持续复制的HBV cccDNA、T细胞失调、B细胞反应不足. 随着乙型肝炎疫苗接种的广泛推广、抗HBV治疗方法的不断更新以及一系列抗HBV药物的临床前/临床试验的推进, HBV的预防和治疗必定会得到极大的改善. 我们需要加大针对非传统病毒靶标的新型治疗方法相关药物研发的投入, 比如宿主靶向药物. 同时, 在慢性乙肝患者中产生并激活HBV特异性B细胞和T细胞是HBV免疫治疗的目标. 随着新型治疗手段的发展, 加上对HBV致病机制的不断探索, 治愈HBV感染将成为可能.

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Summary for “乙型肝炎抗病毒药物研究进展”

Development of antiviral drugs against hepatitis B virus

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Hepatitis B virus (HBV) infection is prevalent throughout the world, with approximately 300 million people chronically infected. HBV infection can cause acute and chronic liver hepatitis. Long-term chronic infection can increase the incidence of liver cirrhosis and hepatocellular carcinoma (HCC). At present, there are two types of approved drugs for chronic HBV infection: nucleos(t)ide analogs (NAs) and interferon α (IFNα), currently pegylated IFNα (PegIFNα). Although these drugs can inhibit viral replication and reduce the risk of liver cirrhosis and HCC, the limitations are obvious: HBV surface antigen (HBsAg) and covalently closed circular DNA (cccDNA) pool cannot be efficiently eliminated. To overcome these limitations, new drugs are required urgently to be designed and developed. In recent years, with the deepening of research on HBV, drugs targeting different targets of viral complex replication mechanisms and interactions with the host immune system have been developed. Drugs targeting viral complex replication mechanisms include HBV entry inhibitors, cccDNA formation and transcription inhibitors, gene editing targeting HBV DNA, HBV specific siRNAs, capsid inhibitors, polymerase inhibitors and HBV secretion inhibitors. Entry inhibitors have been used successfully in treating viral infections. Myrcludex B, a synthetic N-myristoylated lipopeptide derived from HBV preS1 protein, competes with the virus for binding to the solute carrier family 10 member 1 (SLC10A1 or NTCP). This agent prevents HBV and Hepatitis D virus (HDV) infection and in cells and animal models. cccDNA is the transcriptional template of HBV RNAs and is an ideal target for clearing HBV. DNA ligase inhibitors, which can inhibit cccDNA formation and are being developed as anticancer agents, may be tested for treatment of chronic HBV infection, if the side effects are tolerable. The genome editing tool, clustered regularly interspaced short palindromic repeats associated nuclease 9 (CRISPR/Cas9), has many innovative applications in different fields because of its potential for gene therapy. These have provided a clear proof of concept that this approach has the potential to treat or even cure patients with chronic HBV infection. RNA interference technology can be used to manipulate virus gene expression and may be developed for HBV treatment. HBV nucleocapsid formation and pgRNA packaging are critical steps of viral life cycle that may be targeted by antiviral agents. Inside the viral capsid, pgRNA is reverse transcribed into minus-strand DNA by the viral polymerase, with its terminal protein domain acting as a primer for initiation of reverse transcription. HBV polymerase is also a good drug target. Drugs targeting the host immune system include that stimulating the innate immune system, like IFNα, Toll-like receptor (TLR) agonists and stimulating cytoplasmic nucleic acid sensors, and that activating an adaptive immune response, like checkpoint inhibitors, T-cell redirection and therapeutic vaccines. IFN-based therapies are considered as immunomodulatory strategies. TLRs are expressed either at the cell surface or on endosome membranes, where they recognize molecular patterns associated with invading pathogens. Stimulation of TLR1, TLR2, TLR3, and TLR4 on hepatocytes has direct antiviral effects against HBV. The RNA helicases DExD/H-box helicase 58 (DDX58, also called RIG-I) and IFN induced with helicase C domain 1 (IFIH1, also called MDA5) are RNA sensors in the cytoplasm that induce production of cytokines by activating IRF3 and NF-kB. Activation of RIG-I induces an antiviral response against HBV. PD-1 is the inhibitory receptor expressed by most HBV specific T cells, so agents are in development to disrupt the interaction between PD-1 and its ligand, PD-L1. A therapeutic vaccine against HBV needs to overcome mechanisms of HBV-specific immune tolerance. Because natural resolution of HBV infection is accompanied by lifelong T-cell- and antibody-mediated immunity, these vaccines should induce HBV-specific B- and T-cell immune responses. Here, we review recent advances in development of directing-acting antiviral (DAA), host-targeting agents and immunomodulatory therapies. Some of which have entered clinical trials. We also discuss strategies for unbiased high-throughput screens to identify compounds that inhibit HBV and for repurposing existing drugs.

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