

慢性乙型肝炎的功能性治愈

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【摘要】 由于 cccDNA 的持续存在, HBV 感染难以实现完全治愈。目前抗病毒治疗的理想终点是实现 CHB 功能性治愈。现有的抗病毒策略包括核苷类似物和聚乙二醇 IFN- α 在功能性治愈方面的疗效有限。为进一步提高功能性治愈率, 靶向 HBV 生命周期的新型直接作用抗病毒药物和改善宿主免疫应答的免疫调节剂正在开发中。本文总结了 CHB 现有治疗策略以及直接作用抗病毒药物和免疫调节剂的疗效和安全性。

【关键词】 肝炎, 乙型, 慢性; 功能性治愈; 直接作用抗病毒药物; 免疫调节剂; 靶向治疗

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Functional cure of chronic hepatitis B

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【Abstract】 Due to the persistence of cccDNA, it is difficult to achieve complete or sterilizing cure of HBV infection. The ideal end point of antiviral therapy is to realize the functional cure of chronic hepatitis B (CHB). Current HBV therapeutics including nucleoside analogues and peginterferon- α (Peg-IFN- α) have limited efficacy in functional cure. In order to improve the rate of functional cure, new direct-acting antivirals targeting HBV life cycle and immunomodulators to improve host immune response are being developed. In this article, the existing treatment strategies for CHB, the efficacy and safety of direct-acting antivirals and immunomodulators are summarized.

【Key words】 Hepatitis B, chronic; Functional cure; Direct-acting antiviral drugs; Immunomodulators; Targeted therapy

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HBV 感染是世界范围内一个重要公共卫生问题, 影响着全球大约 2.96 亿人, 导致每年约 82 万人死于肝硬化、肝功能失代偿和 HCC 等肝脏疾病^[1]。丙型肝炎的成功治愈启发了人们对 CHB 治愈的探索。然而, 持久存在于肝细胞核中的 cccDNA 是治愈 CHB 患者的最大挑战。即使患者从急性或慢性感染中恢复, 表现为 HBsAg 清除和 HBsAg 血清学转化, cccDNA 仍可持续存在于感染的肝细胞内^[2]。这些患者如果接受癌症化疗或免疫抑制疗法, 将会有潜在的风险^[3]。此外, 整合 HBV DNA 也可持续产生 HBsAg, 特别是在 HBeAg 阴性患者中^[4]。现有的抗病毒策略难以消除 cccDNA 和整合 HBV DNA, 实现 CHB 完全治愈, 国内外专家的共识是以 CHB 功能性治愈为目标, 其定义为: 在完成有限的治疗

过程后, 血清中持续检测不到 HBsAg 和 HBV DNA, 伴或不伴抗-HBs 血清转化^[5-6]。CHB 患者功能性治愈后肝内 cccDNA 水平和 HBV 整合率明显下降, 病毒学复发的风险降低^[7]。因此, 实现血清 HBsAg 清除, 达到功能性治愈是目前理想的抗病毒治疗终点。CHB 患者每年发生自发性 HBsAg 清除率仅有 1.0%~1.2%^[8], 临床上需要有效的抗病毒策略实现更高的 HBsAg 清除率。

一、HBV 生命周期

HBV 属于肝病病毒科, 由脂质包膜和含有部分双链松弛环状 DNA (relaxed-circular DNA, rcDNA) 的蛋白质衣壳组成, rcDNA 由一条完整的负链和一条不完整的正链构成^[9]。HBV 通过细胞膜上牛磺胆酸钠共转运多肽 (sodium taurocholate

co-transporting poly-peptide, NTCP)受体进入肝细胞^[10]。rcDNA 被释放并进入细胞核,在细胞核中被宿主酶修复并转化为高度稳定的 cccDNA^[11],部分 HBV DNA 被整合到宿主基因组中,HBV 整合被认为与 HBsAg 持续表达和 HCC 发生密切相关^[12-13]。cccDNA 是 HBV 所有 RNA 转录的模板,包括前基因组 RNA (pregenome RNA, pgRNA)。此外,cccDNA 也编码 HBV 病毒 X 蛋白(HBx),HBx 是一种非结构蛋白,可以作为转录激活物,在调节病毒基因表达中发挥作用^[14-15]。在细胞质中,病毒核心蛋白与 pgRNA 和病毒聚合酶自我组装形成核衣壳,含有 rcDNA 的成熟病毒衣壳随后被内质网表面蛋白包裹,形成完整的病毒粒子从感染细胞中分泌或运输回细胞核以补充 cccDNA 库^[16]。因此,即使在没有明显的病毒血症情况下,cccDNA 也可持续稳定存在^[17]。消除 cccDNA 或者控制其转录,是寻找 CHB 治愈策略的核心。

二、目前的抗病毒药物

1. 核苷类似物[nucleos(t)ide analogues, NAs]

NAs 可有效减少病毒复制,并已被证明可延缓肝硬化、肝衰竭和 HCC 的进展^[18]。目前治疗 CHB 使用的 NAs 包括富马酸替诺福韦酯、富马酸丙酚替诺福韦、艾米替诺福韦或恩替卡韦^[9],具有较高的抗病毒效力和耐药性屏障。NAs 抑制 HBV 复制,主要靶点是病毒逆转录酶,阻止 pgRNA 形成新的 HBV DNA,对 cccDNA 没有直接影响,使用 NAs 年清除率低于 1%^[19],且 NAs 治疗难以获得持久的免疫学控制,停药后复发率高^[20],因此绝大多数 CHB 患者需要长期甚至终身服用。Berg 等^[21]发现,在实现病毒学控制的患者中停止 NAs 治疗,少数人会获得较强的免疫学应答,实现 HBsAg 清除。原因可能与年龄、性别、种族、NAs 治疗的持续时间、停药时 HBV DNA 滴度以及血清 HBsAg、HBV RNA 以及 HBV 核心相关抗原水平等因素相关。停药时 HBsAg 水平是最佳预测因子^[22],但尚未确定最佳临界值。无论 HBV 基因型如何,停药时 HBsAg > 100 IU/mL 且 HBV 核心相关抗原可检测得到的患者 HBsAg 清除率非常低^[23]。通过 NAs 单药治疗或者 NAs 停药后获得 HBsAg 清除的机会渺茫,CHB 患者应更积极地进行合理有效的抗病毒治疗,实现功能性治愈,降低远期不良结局发生的风险。

2. 聚乙二醇 IFN- α (Peg-IFN- α)

Peg-IFN- α 实现功能性治愈涉及复杂的免疫细胞调控(T 细胞、B 细胞、NK 细胞和 DC 等)^[24-27],最新研究表明 Peg-IFN- α 治疗期间,血清 miR-6126 会升高,抑制 HBV 进入肝细胞,从而减少 cccDNA 合成^[28]。Peg-IFN- α 进一步提升了 CHB 患者优势人群和特殊人群功能治愈率。经 NAs 治疗后,HBsAg 低水平(<1 500 IU/mL)且 HBeAg 阴性的优势患者,接受序贯

PEG-IFN- α 治疗,HBsAg 清除率高达 50%以上^[29]。经 Peg-IFN- α 治疗的非活动性携带者和儿童乙型肝炎患者 HBsAg 清除率分别约为 50%^[30]和 11%^[31],对“不定期”CHB 患者的抗病毒疗效仍在探索中^[32]。Peg-IFN- α 最常见的不良反应是流感样综合征、骨髓抑制、自身免疫病、精神异常、体重减轻和脱发等^[33],限制了其在 CHB 患者中的应用。为进一步探索 Peg-IFN- α 治疗疗效,临床上正在寻找新的标记物,Luo 等^[34]发现 E3 泛素连接酶(TRIM26)通过介导 HBx 泛素化降解来抑制 HBV 复制,IFN- α 可增加 TRIM26 的表达,因此 TRIM26 可作为预测 CHB 患者对 Peg-IFN- α 治疗应答的潜在生物标志物。

三、直接作用抗病毒药物

1. 进入抑制剂

在病毒复制周期中,HBV 通过 NTCP 受体结合进入细胞内,NTCP 是 HBV 和丁型肝炎病毒(hepatitis D virus, HDV)的功能性受体^[10]。布列韦肽(bulevirtide, BLV)能够结合 NTCP 受体,抑制 HBV 与 HDV 进入细胞,已被欧洲药品管理局批准用于治疗慢性 HDV 感染^[35],标志着慢性丁型肝炎治疗的里程碑。BLV 强大的抗病毒活性在 HBV 和 HDV 共感染患者中得到证实,为评估 BLV 与恩替卡韦对 HBV 单感染患者的确切疗效,一项 II 期试验(临床试验编号:NCT02881008)正在进行中。虽然 BLV 可以抑制 HBV 再感染,但对已感染肝细胞内的 cccDNA 没有直接影响,且使用 BLV 长期治疗,可能会影响 NTCP 运输胆汁酸的正常生理功能,使患者出现不同程度的不良反应。

2. 衣壳组装调节剂 [capsid (core)assembly modulators, CAMs]

HBV 核心蛋白在 HBV 生命周期中发挥着重要作用,是 pgRNA 逆转录、病毒粒子形成和 cccDNA 扩增所必需的。CAMs 的主要作用机制是通过干扰 HBV 衣壳组装和 pgRNA 的封装来抑制 HBV 复制^[36]。JNJ-6379^[37]、RO7049389^[38]和 ABI-H0731 (vebicorvir)^[39]等药物在 CHB 患者中具有确切疗效,显示出良好的抗病毒活性。CAMs 单药治疗的 I 期试验显示能有效降低循环 HBV DNA 和 RNA 水平,同时具有良好的安全性,但对 HBeAg 或 HBsAg 水平没有影响^[40-41]。此外,CAMs 与 NAs 联合使用,具有协同抗病毒活性,能明显抑制病毒复制,但对血浆 HBsAg 水平影响甚微^[42]。CAMs 治疗在短时间内难以观察到对 HBsAg 水平有显著影响,在未来的研究中需要评估 CAMs 是否会减少 HBsAg 和 cccDNA。

3. 小干扰 RNA (small interfering RNAs, siRNAs)

血清中高水平的病毒抗原会导致 HBV 特异性 T 细胞免疫功能衰竭和功能障碍^[43]。靶向 HBV RNA 特定位置的 siRNAs

及反义寡核苷酸(antisense oligonucleotides, ASOs)可以减少 HBV 所有病毒转录本,抑制病毒蛋白的翻译,有助于恢复免疫应答,实现 CHB 的功能性治愈。II 期试验中可观察到 VIR-2218^[44]、AB-729^[45]、JNJ-3989^[46]、GSK 3228836^[47]等药物使 HBsAg 水平在短时间内显著降低。此外,ASOs 诱导的 HBsAg 水平降低后,ALT 水平升高,这可能与宿主恢复 HBV 特异性免疫相关^[47-48]。靶向 HBV RNA 能够在短时间内实现显著的 HBsAg 减少,HBsAg 快速降低 2~4 lg 可增强免疫调节剂的疗效^[49]。因此在未来的试验中,应用 siRNAs 或 ASOs 序贯或联合免疫调节剂治疗 CHB 患者,有期望实现功能性治愈。

4. HBsAg 分泌抑制剂-核酸聚合物(nucleic acid polymers, NAPs)

除了传染性病毒粒子分泌外,大量不完整的病毒颗粒也从感染的肝细胞中释放出来,这些由 HBsAg 组成的球形和丝状颗粒,也被称作 HBV 亚病毒颗粒(subviral particle, SVP)。SVP 大量释放,可能会导致免疫耐受和衰竭^[50]。NAPs 可阻止肝细胞分泌 SVP,也可诱导这些 SVP 蛋白体和溶酶体降解^[51]。最近的一项 II 期试验显示,在 HBeAg 阴性患者中评估 REP 2139(NAP)和 REP 2165(NAP)结合 Peg-IFN 和 NAs 治疗的安全性和有效性,13 例(33%)患者实现持续病毒学控制,14 例(35%)患者实现功能治愈^[52]。NAPs 可显著降低病毒抗原载量,但 HBsAg 水平快速下降后,可能会导致 ALT 急性升高,引发肝脏损伤等安全问题,因此需要多中心研究来评估安全性和有效性。

5. 靶向 cccDNA

靶向 cccDNA 的手段主要包括抑制 cccDNA 形成或转录,或诱导其降解。通过 CRISPR/Cas9 系统编辑病毒基因,在细胞培养和动物模型中成功地降低了 HBV DNA 和 cccDNA 水平^[53]。诱导 cccDNA 降解也是 HBV 新药发展的重要方向,研究发现淋巴毒素-β 受体(LTβR)激动剂可刺激肝细胞内 APOBEC3B 的表达,并通过诱导 cccDNA 脱氨基触发其非细胞溶解性降解,且在停止药物处理后仍具有抑制病毒的作用^[54]。此外,HBx 蛋白是 cccDNA 转录所必需的,它可增加宿主限制复合物 Smc5/6 的泛素化水平,诱导其降解,从而增强 cccDNA 转录活性^[55],HBx 蛋白作为抑制 cccDNA 转录的潜在作用靶点目前正在临床前期评估中。基因编辑系统对靶向基因的非绝对特异性识别会导致脱靶效应^[56],它们也会切割染色体整合的 HBV DNA,从而引发染色体 DNA 不可预测重组的可能性^[57]。由于这些潜在的风险,该方法应用于临床上还存在许多限制,需要进一步临床试验验证其疗效及安全性。

四、免疫调节

仅抑制 HBV 复制和降低病毒蛋白表达并不能实现 CHB

功能性治愈,需要免疫调节剂,激活抗病毒免疫应答,修复宿主受损的免疫应答。目前天然免疫调节剂、免疫检查点抑制剂、治疗性疫苗等已在临床中探索。

天然免疫调节剂 Toll 样受体 7(Toll-like receptor, TLR7)激动剂 Vesatolimod(GS-9620)、TLR8 激动剂 selgantolimod(GS-9688)可以增强 NK 细胞和 T 细胞的反应性,诱导免疫介质 IFN-γ 和包括 TNF-α 在内的抗病毒细胞因子产生,但对 HBsAg 水平没有任何影响^[58-59]。虽然有证据表明 TLRs 可使机体免疫激活,但可能需要与其他疗法联合使用。另外天然免疫调节剂视黄酸诱导基因 I(retinoic acid-inducible gene I, RIG-I)激动剂 SB9200 在动物试验中具有良好的抗病毒活性,且与 ETV 联用时,SB9200 诱导的免疫应答可增强 NAs 的效果^[60]。但其临床研究因 1 名试验参与者死亡后被终止。

通过阻断免疫检查点,可以恢复 T 细胞增殖、细胞因子分泌和细胞毒能力,同时降低病毒载量^[61]。程序性细胞死亡蛋白 1(programmed cell death protein 1, PD-1)抑制剂 nivolumab 与治疗性疫苗(GS-4774)联用在 I 期试验中耐受性良好且安全,在 1 例患者中实现了持续的 HBsAg 清除,达到功能性治愈^[62],但免疫检查点抑制剂导致免疫相关的不良事件,包括自身免疫样肝炎不容忽视^[63]。在未来的试验中需要解决的问题是 T 细胞可以恢复到何种程度,以及评估修复 T 细胞导致的组织损伤,需要根据生物标志物或疾病阶段选择最有可能受益于免疫检查点抑制剂的患者群体。

治疗性疫苗用于刺激宿主免疫应答,以恢复 HBV 特异性免疫控制,抑制 HBV 复制,最终减少 HBsAg 水平。目前重组 HBsAg 蛋白疫苗、DNA 疫苗、酵母源疫苗和腺病毒载体疫苗等在临床试验中,但研究未取得令人满意的结果^[64]。基于 HBsAg 和 HBeAg 重组蛋白的 NASVAC 是一个有前景的治疗性疫苗,在 III 期试验中显示出良好的抗病毒效果^[65]。

为了恢复 HBV 特异性 T 细胞免疫功能,基因工程 T 细胞治疗策略在近年来发展迅速。HBsAg-CAR T 细胞在动物模型中具有显著的抗 HBV 活性^[66],此外 Qasim 等^[67]利用重构的 HBV 特异性 T 细胞为治疗 HBV 相关 HCC 提供一种新的方式^[67]。基因工程 T 细胞能否有效抑制 HBV,而不直接损伤肝细胞,还有待进一步研究。HBV 特异性 CAR/TCR-T 细胞治疗方式通过能逆转 CHB 患者 HBV 特异性 T 细胞数量和功能缺陷,从而实现慢性 HBV 感染的功能性治愈。除此之外,靶向 HBV 治疗性抗体也具有临床应用潜力,VIR-3434 是一种全人源单克隆抗体,其作用机制包括抑制病毒进入、抗原提呈、刺激 T 细胞,以及清除 HBsAg,具有良好的耐受性和安全性,并能诱导 HBsAg 快速下降(1~2 lgIU/mL 不等)^[68],未来有

望成为 CHB 治疗策略中的一部分。

五、结语

由于目前的抗病毒药物实现 CHB 患者功能性治愈存在瓶颈,迫切需要研发新的药物获得更高的治愈率。目前多款有前景的药物正在临床试验中,在未来临床实践中,需要 NAs 或 Peg-IFN- α 联合直接作用抗病毒药物及免疫调节剂治疗。目前临床已在开展多种药物联用的抗病毒策略,以抑制病毒复制,减少病毒抗原载量和恢复宿主免疫应答为主导。此外,为了解 CHB 患者的疾病进展,提高患者对抗病毒治疗的应答,改善预后,许多生物学标志物正在探索中^[69-70]。未来需要更多的临床试验,探索实现 CHB 功能性治愈最佳的抗病毒药物组合及生物学评价指标。

利益冲突 所有作者均声明不存在利益冲突

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