

针对共价闭合环状DNA的抗HBV治疗进展

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摘要: 慢性乙型肝炎HBV cccDNA在肝细胞内的持续存在是阻碍慢性乙型肝炎治愈的关键之一。现有抗HBV治疗尚不能作用于HBV cccDNA。随着对于HBV cccDNA生成以及功能基础研究的不断深入, 研究者开始从不同角度设计针对cccDNA的治疗策略: 干扰素- α 以及淋巴毒素 β -受体激动剂通过APOBEC3特异性降解cccDNA, 通过RNA干扰来抑制rcDNA进入细胞核, 通过DNA剪切酶CRISPR-Cas9等靶向降解cccDNA, 通过作用于cccDNA表观遗传学修饰以及通过作用于肝细胞代谢等影响cccDNA功能。这些细胞和动物研究结果提示可降低cccDNA水平或抑制cccDNA的功能, 给HBV彻底清除带来了希望。

关键词: 肝炎病毒, 乙型; 共价闭合环状DNA; 表观遗传学;

Progress in anti-HBV studies targeting HBV covalently closed circular DNA

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Abstract: Persistence of HBV covalently closed circular DNA (cccDNA) in hepatocyte is one of the key obstacles in resolve of chronic hepatitis B. Currently available anti-HBV drugs have no effect on cccDNA. With the further understanding to basic mechanism of HBV cccDNA dynamics and function, now anti-HBV studies targeting different aspects of cccDNA dynamics were developed: specific degradation of cccDNA with APOBEC3 induced by IFN- α and lymphotoxin- β receptor agonists, RNA silencing targeting nucleous localization signal of rcDNA, specific cleavage with CRISPR-Cas9 on cccDNA, epigenetic regulation of cccDNA and regulation of hepatocyte metabolism related to cccDNA function. Primary results of *in vivo* and *in vitro* studies showed inhibition of quantification and function of cccDNA, which bring hope for cure of HBV infection.

Key words: Hepatitis B virus; Covalently closed circular DNA; Epigenetics

就慢性乙型肝炎(chronic hepatitis B, CHB)的抗病毒治疗而言, 随着抗病毒药物不断进展, 尤其是恩替卡韦(entecavir, ETV)和替诺福韦酯(tenofovir disoproxil fumarate, TDF)的应用推广, 使得长期持续抑制HBV复制已成为现实。但现有核苷(酸)类似物[nucleos(t)ide analogs, NAs]仅能作用于HBV聚合酶以阻止HBV链的延长, 对于肝细胞核内的共价闭合环状DNA(covalently closed circular DNA, cccDNA)并无作用^[1]。虽然有研究表明持续抑制HBV可减少细胞内cccDNA含量^[2,3], 但HBV感染的彻底清除仍遥遥无期。近年来不断有针对cccDNA的治疗研究报道, 这些研究从不同角度来设计针对cccDNA的治疗方案且取得初步结果^[4]。现将这些研究综述如下。

1 APOBEC3与cccDNA清除

APOBEC家族是一个脱氨酶家族, 其中多个成员与机体抵抗病毒感染的先天性免疫有关。Lucifora等^[5]研究表明, 干扰素- α (IFN- α)可作用于细胞IFN- α / β 受体从而上调APOBEC3A表达; 而淋巴毒素 β -受体(lymphotoxin- β receptor, LT β R)特异性激动剂可激活LT β R从而上调APOBEC3B表达。上调的APOBEC3A/B均可通过HBV核心蛋白引导而作用于cccDNA脱氨基作用, 从而在细胞内切酶作用下降解cccDNA。虽然有学者对于LT β R激动剂在临床应用的可能不良反应存在质疑^[6], 但该结果无疑为cccDNA的清除研究开辟了全新的方向, 也再次确认了IFN- α 在CHB治疗中的价值。

2 RNA靶向治疗与cccDNA清除

自20世纪90年代以来, RNA干扰已在医学研究与治疗领域广泛应用。HBV核定位信号(nuclear localization signal, NLS)是位于核心蛋白C-末端158~178氨基酸残基的富含精氨酸的序列。NLS与细胞核内cccDNA池的维持有

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关。Li等^[7]首先在HepG2.2.15细胞中证实设计针对HBV核心蛋白C-末端的核定位信号(nuclear localization signal, NLS)的siRNA,可显著抑制cccDNA水平。Li等^[8]进一步在转基因小鼠中证实,其设计的siRNA3可显著抑制cccDNA水平达69%,与之伴随的是HBV rcDNA水平、mRNA及血清标记物等水平均显著下降。

除siRNA外,外部引导序列(external guide sequence, EGS)技术也被用于抗HBV治疗研究。其中Zhang等^[9]与Xia等^[10]均分别设计针对HBV靶位的EGS序列,将其导入细胞中,可引导细胞RNA酶P对特异性HBV序列进行切割,从而起到抑制HBV的作用。但目前尚未见针对cccDNA相关靶位的EGS研究报道。

3 cccDNA转录翻译的表观遗传学调控

肝细胞内cccDNA以微染色体的形式存在,通过调节与其结合的组蛋白的甲基化和乙酰化可以调控cccDNA的转录水平^[11]。其乙酰化水平是由与cccDNA结合的H3/H4组蛋白的乙酰化和去乙酰化来调节的;抑制其去乙酰化可促进HBV复制,而在抗肿瘤治疗中应用去乙酰化抑制剂甚至可以使潜伏的HBV感染再次激活^[12]。干扰素 α 也可通过募集特定组蛋白去乙酰化酶HDAC1与Hsirt到cccDNA来起到抗HBV作用^[13]。Palumbo等^[14]报道通过设计小分子化合物来调节cccDNA微染色体的乙酰化和甲基化可显著抑制HBV的转录和复制,为此类药物研发提供了启示。

4 DNA剪切酶与cccDNA清除

特异性DNA剪切酶用于抗HIV等病毒治疗的研究是近年来研究的热点。可以设计特异性DNA剪切酶通过相关载体进入HBV感染的肝细胞,从而特异性识别cccDNA序列而将其切除。目前关于cccDNA相关剪切酶研究主要见于锌指核酸酶(zinc-finger nuclease, ZFNs)、转录激活因子样效应物核酸酶(transcription-activator-like effector nucleases, TALENs)与CRISPR-cas9系统。Cradick等^[15]报道通过设计HBV cccDNA序列特异性ZFNs,将其转染入相关细胞系,可见ZFN显著降低细胞cccDNA与pgRNA水平。Chen等^[16]报道设计了针对基因A-D型保守序列的TALENs,于细胞实验中证实可显著降低HBeAg、HBsAg水平,并可降低pgRNA与cccDNA水平,该研究进一步于转基因小鼠模型中证实了该结果,并提示TALENs与IFN- α 联合使用存在协同作用。

CRISPR-Cas9系统原本是细菌和古细菌一种不断进化适应的免疫防御机制,它可以利用一段小RNA来识别并剪切DNA以降解外来核酸分子。2013年2月发表于Science的两项研究证明了CRISPR-Cas9系统能在293T、K562与iPS等多种细胞中进行有效的靶向酶切,酶切后进行非同源重组可导致靶基因突变而失去功能^[17,18]。该技术给靶向基因操纵带来了革命性突破,使得多个基因敲除变得极其简单高效。该技术被迅速引入HBV治疗领域^[19-22]。CRISPR-Cas9本身为体细胞基因组编辑工具,而cccDNA类似于人类染色体的定位与结构使其成为CRISPR-Cas9的理想靶标。Seeger等^[21]与Dong等^[22]通过细胞模型与动物模型均证实可以利用CRISPR-Cas9来特异性剪

切cccDNA序列并诱导非同源末端连接进而导致cccDNA失去功能,可显著抑制HBV DNA。

5 肝细胞代谢与cccDNA清除

HBV在肝细胞内可影响糖脂与胆酸代谢,而部分参与糖脂与胆酸代谢的因素可反作用于cccDNA的转录。Zhao等^[23]研究表明人肝细胞核因子4 α 本身作为脂代谢调节因子,可促进HBV pgRNA转录水平,而应用特异性抑制剂抑制人肝细胞核因子4 α 活性可显著降低pgRNA转录水平。过氧化物酶体增殖活化受体 γ 共激活因子-1 α (PGC-1 α)是能量代谢途径中众多转录因子的共激活因子,在能量代谢平衡中起到至关重要的作用,它同时也是HBV转录的共因子。Rechtma等^[24]研究表明,姜黄素可作为PGC-1 α 受体的天然抑制剂,在HBV感染细胞中可抑制cccDNA转录。

6 新型小分子化合物与cccDNA抑制

当前应用的治疗CHB的NAs对于cccDNA均无直接作用。因此研究者试图寻找可直接作用的化合物。Cai等^[25]对85 000种化合物进行筛选,从中筛选出两种化合物CCC-0975与CCC-0346,通过细胞实验证实该两种化合物虽对于细胞内已经形成的cccDNA无作用,但可阻断rcDNA合成cccDNA的过程;研究者希望配合其他NAs,使肝细胞内的cccDNA在无外源性rcDNA补充的情况下而自行耗竭。

7 细胞激酶抑制剂与cccDNA抑制

Lupberger等^[26]在HBV聚合酶上发现了新的核定位信号序列,该位点可被酪蛋白激酶II选择性磷酸化;而该位点磷酸化情况会影响HBV rcDNA入核形成cccDNA。因此研究者应用选择性酪蛋白激酶II抑制剂DMAT可抑制病毒颗粒进入细胞核,从而抑制cccDNA的形成以及形成后的补充。此外如上所述,HBcAg的核定位信号磷酸化会影响pgRNA与HBcAg的组装过程,而Daub等^[27]研究表明丝氨酸/精氨酸蛋白激酶(serine/arginine protein kinase, SRPR)可能参与该位点磷酸化。因此应用SRPK抑制剂可抑制病毒颗粒的组装,从而抑制cccDNA池的补充^[28]。

8 小结

在现有抗HBV药物作用下,HBV感染持续的关键在于cccDNA的存在。由上可知,cccDNA并非稳居于肝细胞核而无懈可击,研究者们已从不同角度设计针对cccDNA的治疗策略。虽然这些研究多处在细胞和动物实验阶段,但也给HBV彻底清除带来了希望。这些进展的根本在于HBV cccDNA形成、代谢与降解的机制研究。只有明确这些机制,才能为相关药物研发提供更多的位点。目前关于cccDNA的相关机制研究的困局在于缺乏合适的动物与细胞模型来模拟HBV自然感染过程^[29],而近年来HBV肝细胞受体发现等研究为构建相关研究模型提供了极大方便^[30]。相信HBV cccDNA研究会不断突破,最终带来HBV的彻底治愈。

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