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评 述 EB病毒发现60周年: 回顾与展望专题

EB病毒的潜伏-裂解感染: 调控机制与靶向策略

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摘要 EB病毒(Epstein-Barr virus, EBV)主要感染人B细胞和上皮细胞. 作为一种疱疹病毒, 它主要有两种感染类型: 潜伏感染和裂解感染. EBV感染绝大部分是潜伏感染, 根据EBV基因表达谱的差异, 可以分为潜伏感染0型、I型、II型、III型. 建立终身潜伏感染是EBV的一大特征, 在潜伏感染阶段EBV在宿主细胞内维持稳定的病毒拷贝数. 裂解感染指病毒在宿主细胞内自主复制、包装、成熟、裂解宿主细胞、释放病毒粒子、再感染其他细胞. 本文综述了EBV潜伏、裂解感染的基因表达谱特征, 代表性分子的功能, 潜伏-裂解感染转换调控的分子机制, 及激活裂解性感染的策略, 旨在为通过利用病毒的潜伏-裂解感染的转换机制进行转化研发提供思路.

关键词 EB病毒, 潜伏感染, 裂解感染

EB病毒(Epstein-Barr virus, EBV)是一种常见的疱疹病毒, 感染全世界绝大部分人口. EBV在机体内有两种感染类型, 一种是潜伏感染, 此时, EBV以“低调”的形式存在于宿主细胞内. 根据感染细胞内的病毒基因表达谱的差异, 可以将潜伏感染分为四种类型, 即0型、I型、II型、III型. 另一种是裂解感染, 此时, EBV DNA在细胞内扩增, 最终合成大量病毒粒子, 从细胞中被释放出来, 以感染周围的其他细胞. EBV的两种感染类型是如何被调控的, 两种感染类型各表达哪些病毒基因, 两种感染类型给宿主带来怎样的后果? 本文将对这方面的研究进展进行综述.

1 EBV潜伏感染-裂解性感染

全球约95%的成年人终身携带EBV[1]. 大多数发展中国家人群初次感染EBV发生在婴幼儿时期, 而发达国家人群初次感染常常延迟到青少年和成人阶段. EBV原发感染通常不引起症状或只产生轻微的症状, 但在B淋巴细胞中建立终身潜伏感染状态. 而在部分青少年中, EBV的原发性感染能够导致急性传染性单

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核细胞增多症(infectious mononucleosis, IM)[2]. 在某些情况下, EBV感染T/NK淋巴细胞进而导致慢性活动性EBV感染(chronic active EBV infection, CAEBV)、EBV相关的嗜血淋巴组织细胞增生症(EBV-associated hemophagocytic lymphohistiocytosis, EBV-HLH)及EBV相关的T/NK细胞淋巴增生性疾病等. EBV的潜伏感染与伯基特淋巴瘤(Burkitt lymphoma, BL)、霍奇金淋巴瘤(Hodgkin lymphoma, HL)、移植后淋巴增殖性疾病(post-transplant lym-phoproliferative disorder, PTPD)、未分化鼻咽癌(nasopharyngeal carcinoma, NPC)、部分胃癌以及多发性硬化症等自身免疫性疾病有直接关系[3,4].

EBV初次感染发生于口咽部上皮细胞, 随唾液脱落后侵入附近淋巴组织, 在B细胞中建立潜伏感染, 也可以从B细胞中再激活进入外周血循环. EBV通过口腔脱落细胞、病毒颗粒在人群中传播[5~7]. 因此, EBV的生命周期包含两个不同的阶段——裂解性感染和潜伏感染. 在裂解性感染阶段, EBV在宿主细胞内进行病毒基因表达、病毒基因组复制以及病毒颗粒的组装, 最终形成具有感染力的子代病毒颗粒并释放到细胞外, 可感染其他细胞或传播给新的宿主. EBV裂解期表达的基因可分为立早期基因、早期基因和晚期基因, 这些基因以级联方式依次协调表达. 首先表达的是两个立早期基因*BZLF1*和*BRLF1*, 编码EBV裂解性感染所必需的转录因子, 共同诱导一系列早期基因的表达, 其中包括参与核苷酸代谢和病毒基因组复制的酶以及激活晚期基因表达的蛋白; 随后, 在病毒DNA复制相关酶的作用下, 环化的病毒基因组通过滚环复制产生头尾相连的线性多聚体DNA, 在末端重复序列处被切割成单个长度的病毒基因组; 最后, 晚期基因编码的衣壳蛋白组装成衣壳并包裹病毒基因组, 核衣壳在病毒编码的核释放复合体作用下转运至细胞质中, 获取一系列的间质蛋白, 并通过出芽方式获取病毒糖蛋白和囊膜, 完成子代病毒的组装[8]. 在潜伏感染阶段, 病毒DNA以共价闭合环状游离体形式存在于细胞核内, 随宿主细胞基因组复制而复制, 并被平均分配到子代细胞中, 维持稳定的病毒低拷贝数. 在潜伏感染的记忆B细胞中, EBV通过限制基因表达及复制的一系列调控机制来逃避免疫系统的监视, 维持病毒潜伏状态, 在体内长期存活.

2 潜伏感染的类型

根据在潜伏感染的细胞中EBV基因表达谱的不同, EBV在感染人群中的潜伏期分为以下4种类型.

潜伏0型, EBV在静息记忆B细胞中的潜伏感染方式. 这个时期病毒的转录程序最为“低调”, 仅表达非编码RNA, 包括EBERs和BARTs, 无蛋白质表达, 常见于既往感染过的健康人B细胞.

潜伏I型, 除非编码RNA(EBERs和BARTs)外, 还表达病毒核抗原EBNA1. EBNA1对EBV基因组的复制和维持非常重要. 该型主要存在于增殖的记忆性B细胞以及伯基特淋巴瘤中.

潜伏II型, 在I型表达的基因之外, 还表达潜伏膜蛋白LMP1和LMP2A/2B. 该型是EBV在生发中心的B细胞中的感染方式, 鼻咽癌、霍奇金淋巴瘤、NK/T细胞淋巴瘤和老年弥漫大B细胞淋巴瘤(diffuse large B-cell lymphoma, DLBCL)中的EBV属于潜伏II型.

潜伏III型: 表达全部潜伏期基因, 包括非编码RNA(EBERs和BARTs), EBNAs(EBNA-1, -2, -3A, -3B, -3C, -LP), LMPs(LMP-1, -2A, -2B). 此类型主要见于高度增殖的B细胞和永生化的淋巴母细胞系(lymphoblastoid cell lines, LCL)、传染性单核细胞增多症、免疫受损患者的淋巴增生性疾病, 如PTLD[1,9].

在潜伏状态下, EBV仅表达(极)少量蛋白, 因此很难引发宿主免疫反应. 通过裂解期诱导剂诱导EBV从潜伏状态进入裂解复制阶段, 使病毒大量复制并表达数十种病毒蛋白, 可诱使免疫系统识别并破坏病毒感染的细胞, 目前逐渐成为一种有效的治疗手段[10].

在B细胞内, 4种潜伏类型都是可能的. EBV在B细胞内的潜伏感染对于病毒的持续存在, 随后在上皮细胞中的复制以及传染性病毒向唾液中的释放是必不可少的. B细胞内的EBV潜伏感染类型通常从III型到II型, 再到I型进行. 不同于B细胞, 上皮细胞感染EBV在正常情况下为裂解感染, 如建立潜伏II期感染可导致恶性肿瘤, 其特征是EBV基因组持续存在、癌基因表达和免疫逃逸.

3 潜伏感染阶段表达的主要病毒基因

EBV潜伏感染阶段主要表达6个核抗原基因EBNAs(*EBNA1*, *EBNA2*, *EBNA3A*/*3B*/*3C*, *EBNA-LP*), 3个

潜伏膜蛋白基因LMPs(*LMP1*, *LMP2A*/*2B*)以及大量非编码RNA(EBERs, BARTs, BHRF1).

3.1 潜伏核抗原EBNAs

EB病毒核抗原1(EBNA1)在所有EBV相关恶性肿瘤细胞中表达, 是潜伏感染期间病毒DNA复制和游离体维持所需的唯一蛋白. EBNA1由N端甘氨酸和精氨酸富含区、甘氨酸和丙氨酸重复序列, 和C端DNA结合域组成. EBNA1通过C端的DNA结合域二聚化并结合到EBV DNA复制起点*OriP*, 调控EBV基因组复制, 维持EBV潜伏感染的稳定性和持久性[11]. EBNA1蛋白的甘氨酸-丙氨酸重复序列可抑制抗原呈递, 有利于免疫逃逸[12]. 此外, EBNA1还可通过甘氨酸和精氨酸富含区与宿主基因组结合, 从而将EBV基因组以环状游离体形式连接到宿主染色体上, 调节在细胞增殖中具有关键功能且与肿瘤发生相关的宿主基因[13]. 目前, EBNA1抑制剂在肿瘤模型中已被证明具有阻止EBV潜伏及肿瘤增殖的作用[14].

EBNA2是潜伏III期表达的核抗原, 是感染后最早表达的病毒基因之一, 可调节其他EBNA和LMP基因的表达以及重编程细胞基因的表达. 作为B淋巴细胞生长转化所必需的蛋白, EBNA2发挥转录因子的功能, 通过与细胞DNA结合蛋白相互作用靶向响应启动子. EBNA2通过DNA结合蛋白CBF1, 反式激活大量病毒和细胞基因的表达. 此外, EBNA2通过与B细胞特异的DNA结合转录因子EBF1共定位或结合细胞转录因子RBP-Jκ, 模拟Notch样信号激活EBV潜伏期启动子的转录[15,16].

EBNA3也是潜伏III期核抗原, 包括EBNA-3A, -3B和-3C. EBNA3A~3C均能与EBNA2竞争性结合RBP-Jκ, 抑制EBNA2的反式激活进而抑制转录[17]. EBNA-3A和EBNA-3C还可通过阻滞细胞从G1期向G2期和(或)G2期向M期转换而抑制细胞生长和增殖[18].

EBNA-LP同样是EBV永生化B细胞的必需基因, 其基因表达由BamHI-W重复序列控制. EBNA-LP可与EBNA2同时表达, 并与之结合作为基因特异的共激活因子, 影响组蛋白乙酰化, 调控染色质结构[19,20].

3.2 潜伏膜抗原LMPs

EB病毒表达两个潜伏期膜抗原(latent membrane protein, LMP)LMP1和LMP2. LMP1是跨膜蛋白, 跨膜区由6个疏水片段组成, 其在细胞膜上的聚合类似于受体交联. 在多种EBV相关肿瘤中能检测到LMP1的表达. LMP1胞内C端的CTAR结构域结合肿瘤坏死因子受体(tumor necrosis factor receptor, TNFR)的胞内信号转导分子包括TRAF和TRADD, 以模拟免疫共刺激分子CD40的功能. CTAR2可通过与接头蛋白和TRAF6相互作用, 导致经典NF-κB信号通路激活、p50/p65复合物形成及c-Jun N端激酶(c-Jun N-terminal kinase, JNK)的活化. CTAR1可激活经典和非经典的NF-κB信号通路产生不同的NF-κB二聚体(如p50/p50, p52/p65和p52/p50), NF-κB可抑制p53依赖的细胞凋亡, 上调*BCL-2*, *MCL-1*及*A20*抗凋亡基因的表达, 促进B细胞增殖. 此外, CTAR1也能激活PI3K, 上调表皮生长因子受体(epidermal growth factor receptor, EGFR)的表达水平[21~24].

LMP2也是跨膜蛋白, 跨膜区由12个跨膜片段组成. LMP2有两种亚型: LMP2A和LMP2B. LMP2A在B细胞中可模拟组成型激活的B细胞受体, 转导下游信号, 促进B细胞的增殖和分化[25]. LMP2A包含多个SH2结构域、一个免疫受体酪氨酸激活基序(immunoreceptor tyrosine-based activation motif, ITAM)和可与Nedd4家族泛素连接酶相互作用的PY基序, ITAM激活可导致Akt的磷酸化和激活、GSK3β抑制、β-catenin信号通路的激活, 阻止上皮细胞分化. LMP2A还可通过ITAM和PY结构域转化上皮细胞, 抑制细胞凋亡, 促进细胞迁移和非依赖性锚定生长[26]. *LMP2B*基因与*LMP2A*相比缺乏*LMP2A*的第一个外显子, 导致LMP2B蛋白缺乏LMP2A胞内N末端结构域的119个氨基酸, 没有信号传导和B细胞受体(B cell receptor, BCR)模拟物的功能.

3.3 非编码RNAs

病毒非编码RNA EBERs主要包括EBER1和EBER2, 分别是由167个和172个核苷酸构成的无帽子、非多聚腺苷酸化的RNA. 在几乎所有的EBV感染的细胞类型中都大量表达, 是EBV感染细胞中最为丰富的RNA, 其结构稳定, 转录产量高, 每个细胞约有10^5个拷贝, 在整个EBV潜伏期均有表达. 即使在EBV基因表达范围最受限的潜伏I型细胞中(如大多数伯基特淋巴瘤), EBERs也始终存在. EBERs可通过多种途径促进宿主细胞的转化、生长和存活, 抑制凋亡, 还可

通过TLR3信号通路诱导I型干扰素、促炎细胞因子的分泌[27]. 潜伏感染期持续高表达的BARTs能够编码大量microRNAs, 能够靶向抑制病毒基因和宿主基因的表达, 例如ebv-miR-BARTs 1/9/16/17靶向LMP1、ebv-miR-BART 22靶向LMP2A, BART-microRNAs也可调控宿主基因, 包括*p53*和*Bcl-2*及宿主免疫相关基因, 如MHC-I类相关链B、Dicer等, 促进肿瘤细胞的增殖和存活, 逃避宿主免疫监视[28~33]. BHRF1 microRNAs仅在潜伏III型表达, 有助于B细胞转化并抑制诱导裂解复制所必需的SUMO化(SUMOylation)过程[34,35]. BARTs和BHRF1 microRNAs均可减弱B细胞受体的信号传导, 有助于抑制EBV的裂解复制[36].

4 裂解性感染期表达的主要病毒基因

只有发生裂解性感染时, EBV才能在细胞间进行传播. 在裂解性感染阶段, 几乎所有的EBV基因都会表达. 在裂解性感染的晚期, 病毒基因组还会出现双向转录.

EBV进入裂解性感染之后, 表达的病毒基因可以分为立早期基因、早期基因、晚期基因. 这些基因以级联方式依次协调表达.

4.1 立早期基因

立早期基因是EBV由潜伏性感染活化为裂解性感染之后最早表达的一组病毒基因, 它们的表达不依赖于其他病毒蛋白的合成. *BZLF1*和*BRLF1*是最重要的立早期基因, 它们编码的蛋白分别叫做Zta和Rta, 这是EBV进入裂解性感染所必需的转录因子. 在处于潜伏感染阶段的细胞中, 表达*BZLF1*基因可以激活裂解性感染[37~40]. BZLF1和BRLF1相互调控, 共同诱导早期基因的转录. BZLF1的活性受到磷酸化的调控[41], 蛋白激酶C磷酸化BZLF1的DNA结合域丝氨酸186位点, 酪蛋白激酶II磷酸化BZLF1的丝氨酸167、173位点. BZLF1激活BRLF1的转录, 后者在宿主转录因子的协助下, 促进病毒基因的转录[42]. *BZLF1*基因的启动子上面可以结合多种转录因子, 如ZEB1, ZEB2结合*BZLF1*的启动子后, 抑制*BZLF1*的转录. SUMO化修饰也参与对*BZLF1*和*BRLF1*活性的调控. 病毒裂解分子LF2诱导BRLF1的SUMO化修饰, 抑制BRLF1的活性.

B细胞分化为浆细胞, 这一分化过程可以导致病毒进入裂解复制. 在这一分化过程中发挥关键调控作用的BLIMP1和XBP-1, 亦是诱导EBV阳性B细胞进入裂解性感染阶段的必需因子[43,44]. 而在上皮细胞中, KLF4和BLIMP1发挥类似的作用[45]. 缺氧、转化生长因子-β和BCR激活, 都可以刺激裂解基因的转录[46].

4.2 早期基因

早期基因约有38个, 主要是与病毒基因组复制有关的基因, 如*BALF5*, 编码DNA多聚酶; *BALF2*, 编码单链DNA结合蛋白; *BORF1*和*BARF2*, 编码核糖核酸还原酶; *BGLF5*, 编码DNA酶; *BXLF1*(TK基因); *BHRF1*, 其编码蛋白是BCL-2的同源物, 具有抗凋亡的功能; *BMRF1*和*BSMLF1*, 编码转录因子; *BSLF1*, *BBLF4*, *BBLF2/3*是引发酶和解旋酶复合体成分, *BKRF3*编码尿嘧啶DNA糖苷酶.

早期基因一般是以核小体结合的DNA为模板而转录, 它们的转录本往往存在一定的位置重叠. DNA多聚酶BALF5可令EBV DNA在进入裂解感染1~2天后就扩增100~1000倍.

裂解期DNA的复制往往伴随着胞核结构的重组, 病毒DNA约占到胞核容积的30%, 导致细胞核呈蜂窝状. 裂解期DNA复制与EBV游离体复制同步开始, 在复制“机构”中, EBV BMRF1构成核心元件, 其周围是新复制的病毒DNA和晚期基因的mRNA[47].

调控裂解感染起始的*oriLyt*位点(顺式作用元件)大约8 kb长. BZLF1结合*oriLyt*后, 使其成为一个超级增强子, 促进其与多个病毒复制蛋白的结合, 包括BALF5, BMRF1, BBLF4, BSLF1[48,49].

核RNA结合蛋白BSMLF1(SM)是裂解复制的一个必需分子. SM结合到EBV转录本的启动子, 增强其稳定性、核输出、剪切、翻译效率[50]. SM和BGLF4(丝氨酸/苏氨酸蛋白激酶)对于晚期裂解基因的表达很关键.

4.3 晚期基因

晚期基因大概有36个, 主要是编码病毒结构蛋白, 在病毒DNA复制之后开始表达, 如*BLLF1*(编码gp350/220)、*BALF4*(编码gp110)、*BXLF2*(编码gp85)、*BCRF1*(其编码蛋白是IL-10同源物).

晚期基因的表达依赖于病毒前起始复合物(viral pre-initiation complex, vPIC), 由6个EBV蛋白组成.

vPIC招募RNA多聚酶II到晚期基因的启动子元件, 驱动晚期基因的转录. 晚期基因的表达还需要病毒DNA复制蛋白、*oriLyt*顺式作用元件的参与.

裂解复制产生的头-尾相连的EBV DNA连环体, 需要被"剪"为合适的大小, 再被"包装"到原壳体. BALF3是病毒末端酶, 它与BGRF1/BDRF1, BFRF1A合作, 剪断EBV DNA连环体. EBV的末端重复序列(terminal repeat, TR)包含538 bp长的序列, 位于线性EBV基因组的两端, 是"包装"信号[51].

蛋白质壳体包裹的EBV基因组以出芽的方式从胞核输出, 在胞浆内进一步成熟、分泌. EBV核输出复合体包括BFRF1和BFLF2. EBV的被膜化(tegumentation)和成熟发生在细胞浆. 病毒颗粒利用细胞内的分泌通路/机制进行运输, 该机制依赖几个关键Rab GTP酶(Rabs8, 10, 11). 成熟的、包装好的EBV病毒粒子通过与细胞膜融合的机制, 被分泌出来[52].

5 潜伏-裂解感染类型的调控

5.1 潜伏和裂解期病毒DNA复制的调节

在潜伏状态下, EBV基因组以闭合环状质粒即游离体的形式存在, 在细胞周期的S期随细胞复制一次, 并在有丝分裂期分离到子代细胞[53]. EBV编码的EBNA1和DNA复制原点OriP在其基因组维持中发挥重要作用[2]. OriP由两簇EBNA1结合序列(即DNA序列)组成, 一个是包含20个30 bp串联重复序列(family of repeat, FR), 另一个是包含4个低亲和力结合位点的二联体对称(dyad symmetry, DS)序列[54]. EBNA1将FR区与染色体结合, 允许复制的病毒DNA保留在子细胞中[54,55]. EBNA1没有解旋酶活性, 这表明病毒DNA复制过程依赖于宿主细胞. 含OriP质粒的复制依赖于细胞因子ORC2和Cdt1[56]. EBNA1-DNA的交联促进复制在OriP处的终止和病毒游离体的维持[57]. 宿主去泛素酶USP7与EBNA1结合则能抑制EBV DNA复制[58]. 本实验室研究表明, EBNA1还可以结合亲环素A(cyclophilin A, CYPA), 当CYPA过表达时, EBNA1-CYPA能拮抗EBNA1-USP7的作用, 从而促进病毒DNA的复制[59]. 而CYPA在EBV感染细胞中表达上调, 这种上调依赖于LMP1激活的NF-κB, CYPA可以激活AKT/mTOR/NF-κB正反馈环路[60]. OriP还能与染色质重塑蛋白SNF2h和染色质修饰蛋白HBO1和HP1相关的起点识别复合物(origin-recognition complex, ORC)成分相互作用[61].

与潜伏期的复制不同, EBV的DNA裂解性复制在*oriLyt*启动多轮复制, 且复制过程对EBV编码的蛋白质有更大的依赖性[62]. 有7个必需的EBV DNA复制核心基因, 包括*BZLF1*, *BALF5*, *BMRF1*, *BALF2*, *BBLF4*, *BSLF1*和*BBLF2/3*. BZLF1(Zta)可作为oriLyt结合蛋白发挥作用[63]. 除BZLF1外, 所有蛋白都可以在复制叉上一起合成串联EBV基因组的前导链和滞后链[64]. 在裂解复制循环诱导后不久, 病毒DNA被扩增, 子代DNA具有较少的负超螺旋和核小体, 可能优先被DNase I切割, 从而为滚环复制提供理想的模板DNA.

5.2 EBV潜伏期维持的调节

在潜伏期中, 病毒表达很少的蛋白和非编码RNA, 并通过劫持或激活宿主细胞多因子及其作用, 逃避宿主免疫监视, 同时影响宿主细胞的增殖和凋亡等生物学特性, 促进病毒与细胞的共存, 并发挥一定的致瘤作用[65]. EBV病毒潜伏期的破坏是由BZLF1(在B细胞和上皮细胞中)或BRLF1(仅在上皮细胞中)的表达而诱导的[63,64].

STAT蛋白家族转录因子在调节细胞生理过程, 如增殖、分化、凋亡和血管生成中发挥重要作用. EBV可以通过STAT3激活半胱天冬酶9和7, 同时避免EBV感染的细胞死亡, 并促进细胞增殖[66]. EBV和KSHV这两种致瘤疱疹病毒在感染原代细胞后, 可激活STAT3. STAT3在抑制对EBV癌基因触发的复制应激的DNA损伤反应(DNA damage response, DDR)中起关键作用, 从而促进B细胞增殖, 最终建立潜伏期[67]. EBV还可以通过激活STAT3阻止Chk1的磷酸化, 从而抑制S期内细胞周期检查点的激活, 有助于维持B细胞中病毒的潜伏期[68].

转录因子E2F1参与细胞周期、DNA复制、修复、细胞有丝分裂和细胞命运的调控[69]. EBNA1的甘氨酸-丙氨酸重复序列可以触发mRNA翻译应激, 从而激活PI3Kδ, 同时使MDM2蛋白稳定, 诱导MDM2与E2F1的mRNA结合, 促进E2F1翻译, 进而激活MYC和细胞增殖, 以维持EBV病毒的潜伏状态[70]. 用PI3Kδ抑制剂Idelalisib(CAL-101)则可抑制E2F1和MYC水平并导致EBNA1诱导的B细胞淋巴瘤中的细胞裂解死

亡[71]. E2F1还可以和EBNA3C结合形成复合物, 使E2F1被泛素-蛋白酶体系统降解, 这对于抑制DNA损伤诱导的E2F1介导的细胞凋亡至关重要, 从而也有利于EBV的潜伏感染[72].

EBV潜伏基因的表达受到其基因组DNA甲基化的严格调控[73]. 地西他滨(一种DNA甲基化特异性抑制剂)可以使p73和转录因子RUNX3的启动子区域去甲基化、表达上调, 也可以上调BZLF1, 从而诱导EBV由潜伏向裂解转换[74].

此外, MYC可以结合*oriLyt*, 并抑制其与*BZLF1*启动子的结合和复制循, 敲低*MYC*或*MYC*表达的重要调节因子会导致EBV重激活[75]. 本实验室还发现EBNA1结合BRD7, 进一步通过染色质重塑调节原位和易位*MYC*基因的转录, 从而促进EBV潜伏期的维持[76]. 这也说明EBV主动参与调节潜伏-裂解的调控.

在EBV阳性鼻咽癌和胃癌中, 上皮分化标志物ΔNp63α可能诱导BARF1表达, 这解释了在没有裂解激活的情况下BARF1的表达[77]. ΔNp63α还可通过抑制Zp促进EBV潜伏期. 另一种p63基因剪接变体TAp63α在B细胞淋巴瘤中也可以抑制EBV病毒的裂解激活, 从而促进恶性肿瘤中的EBV潜伏感染[78]. EBNA1及EBV编码的miRNA还可直接抑制p53的表达[79,80], 而EBV对p53的抑制可阻断p53响应性长链非编码RNA IGFBP7-AS1的表达, 从而促进细胞增殖和抑制细胞凋亡, 这也有利于EBV的潜伏感染和致瘤作用[81].

转录因子YY1在EBV感染的B细胞中也能负调控BZLF1和BRLF1转录, 在维持EBV潜伏期中起重要作用[82,83]. *BRLF1*基因启动子中YY1结合位点的突变, 能显著促进BRLF1的转录, 这也说明YY1负向调控BRLF1的表达[84]. 在Burkitt淋巴瘤细胞HH514-16BL中, 使用组蛋白脱乙酰酶抑制剂丁酸钠(NaB)、古抑菌素A(TSA)或DNA甲基转移酶抑制剂AzaCdR均可诱导EBV重激活, 丙戊酸则可选择性地刺激一些细胞基因的表达, 如*MEF2D*, *YY1*和*ZEB1*, 而这些基因可以抑制EBV的裂解激活, 进而维持潜伏感染[85].

一些天然免疫分子参与调节EBV潜伏-裂解复制. IFIT3在EBV潜伏感染细胞中表达上调, 当诱导EBV进入裂解期, IFIT3表达进一步升高, 并通过促进天然免疫对抗裂解复制[86]. 因此, IFIT3趋向于维持EBV潜伏感染.

5.3 EBV裂解期的调节

转录因子FOXO3促进EBV病毒裂解感染[87], 但它常会被宿主和病毒miRNA抑制[88]. 转录因子ZEB1和ZEB2通过结合Zp抑制BZLF1基因转录和表达, 抑制裂解感染; 细胞miR-200b和miR-429可通过下调ZEB1/2诱导EBV裂解复制[89]. 这种拮抗作用有利于EBV潜伏-裂解期的平衡调节.

Rta蛋白可以与MCAF1的相互作用, 激活转录因子Sp1介导的基因转录[90]. 上游转录因子1(upstream transcription factor 1, USF1)也与EBV中DNA聚合酶的启动子激活相关. LMP1启动子的活性可被USF1和USF2a的过表达上调, 其反式激活被MAX和MAD1抑制[91]. USF1还可以与E1(BRLF1启动子Rp中的一个E-box激活位点)结合, 进而促进EBV病毒的重激活[92].

EBNA2的反式激活结构域与转录因子TFIIH相互作用, 激活宿主和病毒基因转录[93]. EBV裂解期早期表达的SM核蛋白招募TFIIH的亚基XPB, 进而结合15个EBV晚期裂解基因的启动子, 促进EBV的裂解复制[50].

YAP/TAZ与溴结构域蛋白4(bromodomain protein 4, BRD4)结合, 诱导EBV的裂解激活[94]. 使用YAP/TAZ激活剂溶磷脂酸可以诱导EBV的重激活. EBV感染的B细胞中YAP/TAZ处于很低水平, 这可能部分解释了EBV在B细胞中更趋向于维持潜伏感染状态[95].

在缺氧培养条件下, Zta的表达上调, 有助于诱导EBV的裂解复制[96]. 缺氧诱导因子HIF-α被可以直接结合Zp启动子, 促进EBV重激活[97]. 缺氧诱导的病毒重激活可能与相关肿瘤在缺氧条件下的代谢及致瘤作用有关联, 值得深入探讨.

表观遗传修饰与病毒感染的关系非常密切[98,99]. EBV从潜伏期转化至裂解期, 病毒和细胞发生一系列表观遗传修饰变化. EBV处于潜伏期时, 病毒基因高度甲基化, 进入裂解期后, 一些重要的病毒基因发生去甲基化, 在感染完成后这些基因再次甲基化, 从而沉默病毒基因的表达, 又进入潜伏期[100]. EBV潜伏期时PAX5和Oct-2与Zta蛋白结合, 抑制Zta和ZRE(Zta反应元件)结合, 而BCR刺激和浆细胞分化使这两个因子沉默, 失去作用[101]. 激活的Zta蛋白倾向于和CpG甲基化的ZREs结合, 同时ZRE与CpG甲基化的DNA更容易结合, 使得Zta蛋白可以激活在潜伏期被甲基化修饰所沉默的裂解基因[101,102].

EBV感染可导致细胞基因组异常甲基化[103]. 一方面, LMP1可以通过JNKs信号途径促进DNMT1(DNA甲基转移酶1)的表达, 促进宿主基因组的甲基化; 另一方面, EBV感染会降低TET2(甲基胞嘧啶双加氧酶2)的表达, 抑制细胞去甲基化的途径[104].

一般情况下, *BZLF1*基因被抑制性组蛋白修饰(如H3K27me3, H3K9me3, H4K20me3)所沉默. 当某些因素导致组蛋白去甲基化或乙酰化时, 将促进*BZLF1*基因活化, 促进EBV由潜伏期转化为裂解期[105]. H3K27me3等组蛋白修饰对*BZLF1*基因启动子(Zp)的抑制作用不太强, “清除”这些修饰之后, 使得Zp可以迅速激活; 但也不太弱, 刚好能抑制Zp, 而这对EBV潜伏期的维持至关重要[99,100].

6 EBV裂解诱导疗法

EBV可以在人体中建立终身潜伏感染, 在一定条件下, EB病毒能够重新激活并进入几乎表达所有病毒基因的裂解复制期. EBV在裂解期复制其病毒基因组并产生具有再次感染能力的子代病毒. EBV的裂解性复制通常会导致宿主细胞死亡和子代病毒粒子的释放[106].

EBV裂解诱导疗法是指通过诱导EBV感染进入裂解复制状态[107], 不仅可以导致宿主肿瘤细胞的死亡, 还能通过激活机体的免疫应答而发挥抗肿瘤作用, 是针对EBV阳性肿瘤的一种有效靶向治疗策略(图1). 一方面, EBV阳性肿瘤中几乎所有的肿瘤细胞均被EBV感染, 并且病毒均处于潜伏感染期. 诱导EBV进入裂解复制期会产生大量子代病毒, 从而导致其宿主细胞(肿瘤细胞)的破裂、分解和死亡[108]. 另一方面, 在潜伏感染期, EBV DNA高甲基化控制着潜伏期程序的选择, 并进一步抑制裂解基因的表达和多种病毒蛋白的免疫原性, 使得机体免疫系统对病毒难以监控、识别, 有助于EBV阳性肿瘤的免疫逃逸[109]. 而在裂解复制期, EBV几乎表达所有病毒基因以满足其基因组复制的需求, 更多EBV抗原分子暴露给机体免疫系统; 病毒抗原被抗原提呈细胞捕获, 诱导机体产生强烈的免疫反应. 促进EBV阳性肿瘤中的EBV从潜伏感染期快速转换进入裂解复制期, 有助于诱发机体的特异性免疫应答, 增强免疫系统对EBV阳性肿瘤细胞的清除作用[110,111].

多种类型的化合物被开发出来用于诱导EBV感染进入裂解复制期. 佛波酯(TPA)可激活EBV裂解复制, 导致Raji细胞中的染色体DNA片段化[112]. 阿霉素(Doxorubicin)、吉西他滨(Gemcitabine)、环磷酰胺(Cyclophosphamide)、顺铂(Cisplatin)、硼替佐米(Bortezomib)和氟尿嘧啶(5-fluorouracil)是能有效诱导EBV裂解感染的传统化疗药物[113~116]. 影响DNA甲基化和组蛋白去乙酰化的小分子抑制剂也能诱导EBV进入裂解复制期, 例如, 5氮杂胞嘧啶核苷(一种DNA甲基转移酶抑制剂), 丁酸钠、丙戊酸、异羟肟酸等组蛋白去乙酰化酶抑制剂[117~119]. 秋水仙碱和长春花碱等微管解聚化合物可以通过激活PKC, JNK和p38信号通路, 从而激活EBV裂解复制[120]. H_2O_2, 1-甲基-3-硝基-1-亚硝基(MNNG)等活性氧诱导剂可激活p53[121], p53随后与Zp和Rp中的Sp1结合元件结合, 激活EBV裂解复制[122]. 缺氧诱导因子1(hypoxia-inducible factor-1, HIF-1)与Zp上的缺氧反应元件基序结合能激活ERK1/2信号通路, 从而诱导缺氧状态, 重新激活EBV进入裂解复制期[97,123]. 铁螯合剂, 如去铁胺、Dp44mT和一种称为C7的新型化合物被发现可以稳定HIF-1, 导致EBV的裂解复制[123].

EBV被激活进入裂解性感染后, 产生BGLF4(丝氨酸/苏氨酸蛋白激酶)[3], 磷酸化阿昔洛韦和更昔洛韦等抗病毒核苷类药物, 将其转化为细胞毒性形式, 从而对EBV阳性细胞产生特异性杀伤作用[124]. 因此, 阿昔洛韦和更昔洛韦等抗病毒核苷类药物也有助于EBV裂解诱导疗法[10,119].

除上述化合物外, anti-IgG, BCR和TGF-β等生物刺激因子也可以诱导激活EBV裂解性感染. 在Akata细胞系中, 将BCR与抗人IgG抗体交联可以有效诱导EBV的裂解复制[125]. BCR的参与能通过激活B细胞中EBV的*BZLF1*基因表达诱导EBV进入裂解复制[126~128]. T细胞分泌的细胞因子TGF-β可通过激活某些伯基特淋巴瘤细胞系的*BZLF1*基因表达诱导EBV进入裂解复制[129~131].

目前为止, EBV裂解诱导疗法主要处于实验室研究阶段, 只有少数EBV阳性肿瘤患者在临床上接受过裂解诱导治疗, 且多以联合用药的方式进行. 有研究入组了15例常规治疗无效的EBV阳性淋巴瘤患者[132], 采用HDAC抑制剂丁酸精氨酸和抗病毒核苷类药物更昔洛韦进行联合治疗, 其中, 有4例患者痊愈, 6例患者

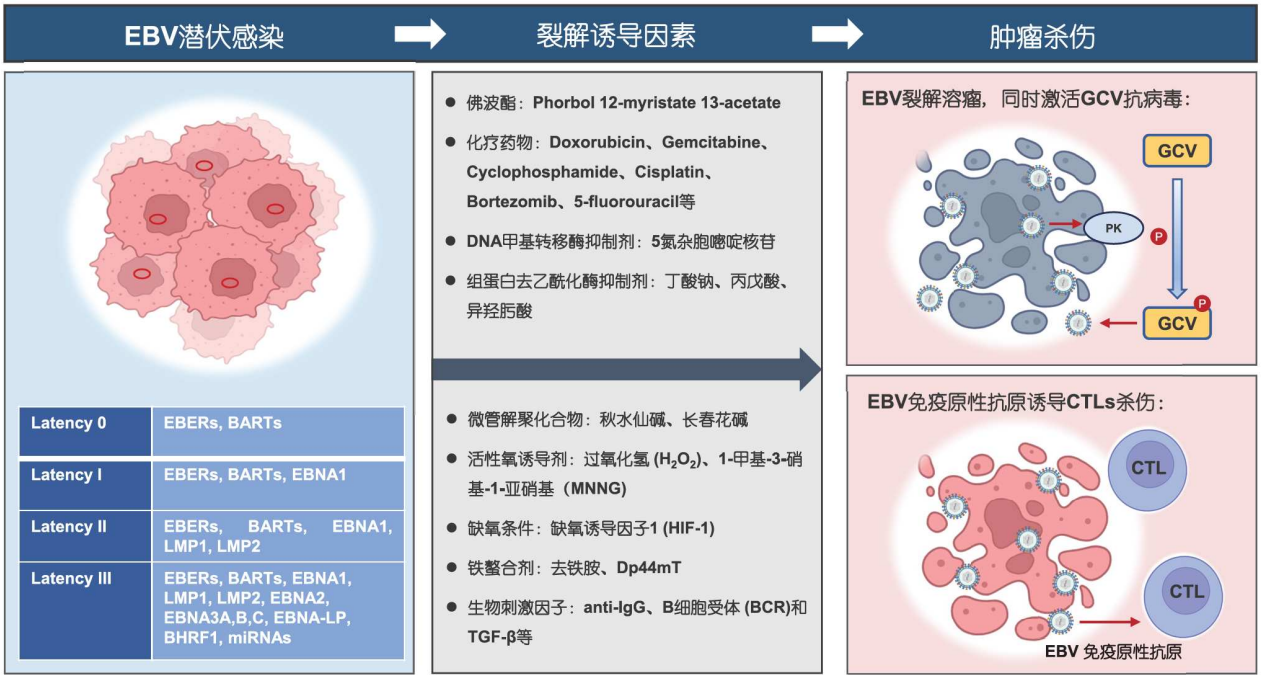

图 1　EBV裂解诱导疗法简述. 根据在潜伏感染的细胞中EBV基因表达谱的不同, EBV在感染人群中的潜伏期分为4种类型: 潜伏0型, 潜伏I型, 潜伏II型和潜伏III型. 在EBV裂解诱导因素的作用下, EBV感染进入裂解复制状态. 一方面, EBV进入裂解复制期产生大量子代病毒, 导致其宿主细胞(肿瘤细胞)的破裂、分解和死亡. 同时EBV裂解表达的病毒蛋白激酶将以磷酸化的作用激活抗病毒核苷类药物, 例如, 更昔洛韦(GCV). 另一方面, EBV进入裂解期几乎表达所有病毒基因以满足其基因组复制的需求, 更多EBV免疫原性抗原分子得以暴露于机体免疫系统, 诱导机体的特异性免疫应答, 扩大免疫系统对EBV阳性肿瘤细胞的清除作用(PK: 病毒蛋白激酶; GCV: 更昔洛韦; CTL: 细胞毒性T细胞)

Figure 1　Brief introduction of EBV lytic reactivation therapy. According to the different EBV gene expression profiles in latently infected cells, EBV has four gene expression profiles during latent infection in population: type 0, type I, type II, and type III. Upon the activation by lytic inducing factors, EBV enters a lytic replication state. On the one hand, EBV enters the lytic replication phase and produces a large number of progeny viruses, resulting in the disruption, decomposition and death of its host cells (tumor cells). At the same time, viral protein kinases expressed in EBV lytic phase, will phosphorylate and activate antiviral nucleoside drugs such as ganciclovir (GCV). On the other hand, EBV expresses almost all virus genes to meet the needs of virus genome replication, and more EBV immunogenic antigen molecules can be exposed to the immune system, inducing specific immune response and expanding the elimination of EBV positive tumor cells by the host immune system. PK: viral protein kinase; GCV: ganciclovir; CTL: cytotoxic T cells

的病情得到部分缓解. 使用吉西他滨、丙戊酸和更昔洛韦对3例常规治疗无效的晚期EBV阳性鼻咽癌患者进行联合治疗, 治疗过程中3例患者的外周血中均检测到病毒DNA含量明显升高, 肿瘤得到有效控制, 患者病情得到基本稳定[133].

过去几十年来, 尽管有许多研究围绕EBV裂解诱导疗法和EBV裂解诱导药物展开, 认为诱导EBV进入裂解复制期是一种靶向EBV阳性肿瘤的治疗策略, 但在EBV裂解诱导治疗过程中始终存在病毒传播难以控制的担忧. 因此, 将不同类别的裂解诱导化合物进行组合治疗, 探索临床上其他的可用药物, 或开发新型的多肽药物、小分子抑制剂和基因编辑药物等作为辅助治疗, 将成为有前景的发展方向.

7　总结与展望

通常情况下, EBV主要以潜伏感染的形式持续存在于宿主体内, 在受到裂解诱导因素的调控下会进入到裂解感染状态. EBV在特定肿瘤类型中的持续存在为开发病毒特异性的肿瘤靶向治疗提供了可能. 随着人们对EBV潜伏-裂解性感染机制和病毒基因表达调控机理的理解不断加深, EBV潜伏感染状态的建立和裂解再激活如何调控EBV相关恶性肿瘤的发生发展, 正在不断被揭示, 基于EBV的更多治疗靶标也在被鉴定出来. 相信在不久的将来, 基于潜伏-裂解调控机制而开发的靶向药物会逐渐走向临床, 成为EBV相关性疾病的重要治疗手段.

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Regulation of latency-lytic infection of EBV and the its therapeutic strategy

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Epstein-Barr virus (EBV) mainly infects human B cells and epithelial cells. As a herpesvirus, it has two main types of infection: latent infection and lytic infection. Epstein-Barr virus infection is predominantly latent. According to the difference of EBV gene expression profile, latent infection can be divided into type 0, type I, type II and type III. The establishment of lifelong latent infection is a major feature of EBV. During the latent infection stage, EBV maintains a stable number of virus copies in the host cells. Lytic infection means that the virus replicates, packages, matures, cleaves host cells, releases virions, and then infects other cells. This review describes the characteristics of gene expression profile of EBV latent and lytic infection, the function of representative molecules, the regulation mechanism of latent and lytic infection switch, and the targeting strategy and clinical value of reactivating lytic infection.

Epstein-Barr virus, **latent infection**, **lytic infection**

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