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评 述 EB病毒发现60周年: 回顾与展望专题

EBV非编码RNA的功能及其机制

何江[1], 熊炜[2], 李欣[3], 孙仑泉[1,4*]

1. 中南大学湘雅肿瘤医学中心, 分子放射肿瘤学湖南省重点实验室, 湖南省肿瘤精准医学国际科技合作基地, 长沙 410008;
2. 中南大学肿瘤研究所, 长沙 410078;
3. 南方医科大学深圳医院, 深圳 518000;
4. 国家老年疾病临床研究中心(湘雅医院)老年肿瘤研究所, 长沙 410008
* 联系人, E-mail: lunquansun@csu.edu.cn

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摘要 EB病毒(Epstein-Barr virus, EBV)属于γ疱疹病毒亚科, 在人群中广泛流行, 与鼻咽癌、胃癌和淋巴瘤等多种恶性肿瘤的发生密切相关. 然而, EBV致瘤的分子机制仍不清楚. EBV非编码RNA包括EBV长非编码RNAs (EBV long non-coding RNAs, EBV lncRNAs)、EBV编码小RNAs(EBV-encoded small RNAs, EBERs)和EBV微小RNA(EBV microRNAs, EBV miRNAs). 通过与潜在靶基因相互作用, EBV非编码RNA在病毒复制与潜伏、肿瘤细胞存活、增殖、转移和免疫逃逸等方面扮演重要角色, 是近年来的研究热点. 本文就EBV lncRNAs, EBERs和EBV miRNAs在EBV相关肿瘤中的功能及其介导的信号通路进行综述, 并对该领域存在的问题进行讨论, 以期为靶向EBV非编码RNA的临床应用提供新思路.

关键词 EBV, EBV非编码RNA, EBV长非编码RNA, EBV编码小RNA, EBV编码微小RNA

EB病毒(Epstein-Barr virus, EBV)属于γ疱疹病毒亚科, 是一种大型双链DNA病毒, 于1964年在伯基特淋巴瘤中首次被发现, 至今已60周年. 该病毒现在人群中广泛流行, 超过90%的人口被感染, 其也是第一种被明确的人类肿瘤病毒, 与鼻咽癌、胃癌和各种类型淋巴瘤(如伯基特淋巴瘤、霍奇金淋巴瘤和NK/T细胞淋巴瘤)的发生有关[1,2]. 除了肿瘤, EB病毒还与非恶性疾病发生有关, 如传染性单核细胞增多症、口腔毛状白斑病、系统性红斑狼疮和多发性硬化症.

EBV非编码RNA包括长非编码RNAs (EBV long non-coding RNAs, EBV lncRNAs)、EBV编码小RNAs (EBV-encoded small RNAs, EBERs)和EBV微小RNA (EBV microRNAs, EBV miRNAs). lncRNA是一类长度超过200个核苷酸且缺乏蛋白质编码能力的RNA[3], 与细胞增殖、凋亡、迁移、侵袭和分化等多种细胞生命活动密切相关[4~6]. lncRNA虽然在结构上与mRNA相似, 但与常见于细胞质的mRNA不同. lncRNA可以在各种亚细胞区室中检测到, 如细胞核和细胞质[7,8]. 此外, lncRNA的定位具有组织特异性, 比mRNA更保守, 其包含的 miRNA 结合序列可作为 miRNA 结合位点而劫持miRNA, 导致靶向mRNA的miRNA减少. 据报道, lncRNA通过这种劫持miRNA的方式调控众多基因

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表达, 从而影响细胞稳态[9,10]. 相似地, EBV lncRNA也可以竞争性地与miRNA结合抑制其靶向mRNA的能力, 从而调节宿主基因表达, 促进EBV相关肿瘤发生[11~13].

EBERs包括EBER1和EBER2, 均由RNA聚合酶III (pol III)转录的非PolyA的非编码RNA, 分别包含166和172个核苷酸. 研究发现, EBERs是EBV潜伏感染细胞中最丰富的病毒转录本, 其内部核苷酸呈现高度互补, 排列成部分双链结构, 其中包含短而稳定的茎环. 这种空间结构使它们能够与一些特定的蛋白相互作用, 如dsRNA依赖性蛋白激酶(PKR), 从而形成RNA-蛋白质复合物. 有研究证实, 淋巴瘤细胞中EBER对PKR诱导的细胞凋亡具有抗性[14]. EBER的这种抗凋亡能力促进了EBV相关肿瘤的发生. 此外, EBER也能通过调控RIG-I和TLR3介导的炎性信号, 促进鼻咽癌发展[15].

除EBER外, 研究发现, EBV至少还编码44个高度保守的miRNA, 这是迄今为止在人类病原体中鉴定出的最多的miRNA. 那些miRNA转录本可分为两个亚群, 即miR-BARTs和miR-BHRF1s. 这两组编码miRNA的基因定位于EBV基因组两个不同区域, 即产生4个成熟miRNA的BHRF1区域和产生40个成熟miRNA的BART区域[16~18]. 迄今为止, 虽然EBV miRNA在维持病毒潜伏感染和促进肿瘤免疫逃逸等方面发挥重要作用, 但许多EBV miRNA功能仍然未知[16].

本综述重点概述了EBV编码的lncRNAs, EBERs和miRNAs在EBV相关肿瘤中涉及的恶性生物学表型和信号通路(图1), 并针对该领域存在的问题进行了讨论.

图 1　EBV非编码RNA介导的恶性肿瘤生物学表型及信号通路. EBV非编码RNA和包括免疫逃逸、肿瘤促炎反应、诱导新生血管、逃逸生长抑制因子、侵袭转移、细胞死亡抵抗、改变表型可塑性和持续增殖在内的8个肿瘤特征密切相关

Figure 1　The tumor biological phenotypes and signaling pathways meditated by EBV non-coding RNA. EBV non-coding RNA is closely associated with the eight hallmarks of cancer, including immune evasion, tumor-promoting inflammation, induction of angiogenesis, evasion of growth suppressors, activation of invasion and metastasis, resistance to cell death, unlocking phenotypic plasticity, and sustaining proliferation

1　EBV长非编码RNAs

包括环状RNA (circular RNA, circRNA)家族在内的长度超过200 nt且没有编码蛋白能力的病毒非编码RNA, 称为病毒长非编码RNA[8]. EBV长非编码RNA包括线性lncRNA和闭合共价circRNA[19,20]. 目前研究表明, EBV lncRNA和circRNA参与了EBV潜伏、复制和裂解以及肿瘤增殖、侵袭、迁移和免疫逃逸[21~27](表1), 但很多EBV lncRNA和circRNA功能仍不清楚.

1.1　EBV lncRNA

lncRNA主要是RNA聚合酶II (Pol II)的转录和剪接产物. 它们通常有5′帽子, 并且3′末端可能包含PolyA尾巴. 迄今为止, 发现EBV至少包含两个编码lncRNA的区域. 一个位于BamHI-H左阅读框(BHLF1)区域, 其编码的lncRNA称为BHLF1 lncRNA. 进一步研究表明, *BHLF1*是一种潜伏期开始表达的裂解循环基因, 长度为1980 bp且富含G, 在EBV裂解期大量表达[28,29].

最初, *BHLF1*是在EBV感染细胞多核糖体部分被发现的长度为125 bp的重复序列. 尽管它有一个开放阅读框(open reading frame, ORF), 但没有鉴定到相应蛋白, 表明*BHLF1*基因编码的产物可能是lncRNA[30]. 研究表明, BHLF1作为功能性lncRNA存在富含G的RNA序列, 可以形成稳定的RNA-DNA杂交体, 并与DNA单链结合蛋白BALF2结合, 启动裂解复制[31]. 此外, BHLF1 lncRNA在RNA结合蛋白募集到EBV诱导的核结构过程中发挥结构和支架作用[32]. 虽然BHLF1 lncRNA功能尚不清楚, 但许多研究提示这些lncRNA参与了病毒增殖、复制和裂解以及相关肿瘤的发生发

表 1 EBV非编码RNA的功能和相关疾病汇总

Table 1 The summary of EBV non-coding RNA in malignant diseases

非编码RNA	功能	机制	肿瘤类型	参考文献
circRPMS1	促进细胞增殖、迁移和侵袭 抑制细胞凋亡	上调METTL3表达	胃癌	[25]
	抑制鼻咽癌细胞增殖和EMT 诱导细胞凋亡	通过海绵作用竞争性抑制miR-31, miR-203 和 miR-451	鼻咽癌	[49]
circLMP2A	维持肿瘤干细胞干性	靶向 miR-3908/TRIM59/p53 轴维持肿瘤干细胞干性	胃癌	[26]
circBART2.2	肿瘤免疫逃逸	上调PD-L1表达并抑制T细胞功能	鼻咽癌	[27]
BART lncRNA	促进肿瘤发生	下调肿瘤抑制因子RASA1	胃癌	[33]
		调控组蛋白活性或DNA甲基化表型	胃癌	
		甲基化CpG岛启动子, 导致特定基因沉默	胃癌	
		直接或间接下调UPR主要转录因子	胃癌, 鼻咽癌	
		上调Aiolos参与表观遗传修饰调控	鼻咽癌	[37,41,42]
miR-BART5-5p	肿瘤免疫逃逸	通过靶向PIAS3并激活STAT3, 上调PD-L1的表达	胃癌	[90]
	抑制肿瘤细胞凋亡	靶向促凋亡PUMA	鼻咽癌 胃癌	[76]
miR-BART11 miR-BART17-3p	肿瘤免疫逃逸 肿瘤免疫逃逸	通过靶向FOXP1和PBRM1, 促进PD-L1表达	鼻咽癌	[91]
miR-BART13	抑制鼻咽癌细胞增殖	抑制NF-κB信号	鼻咽癌	[63]
miR-BART11-5p	促进胃癌细胞增殖 抑制肿瘤细胞凋亡	直接靶向Rb和p21	胃癌	[64]
miR-BART2-5p	促进胃癌细胞增殖 抑制肿瘤细胞凋亡	靶向肿瘤抑制因子RB和细胞增殖抑制基因p21	胃癌	[64]
	促进鼻咽癌转移	靶向Rho信号负调节因子 RND3	鼻咽癌	[86]
miR-BART10-3p	促进鼻咽癌细胞增殖	靶向ALK7通路	鼻咽癌	[65]
	促进胃癌细胞增殖	下调DKK1表达, 激活WNT通路	胃癌	[66]
	促进肿瘤细胞迁移和侵袭	靶向DKK1诱导 EMT		[66]
miR-BART20-5p	促进胃癌细胞增殖	下调BAD 表达	胃癌	[67]
miR-BART4	促进胃癌细胞增殖	下调肿瘤抑制因子PTEN表达	鼻咽癌	[68]
miR-BART6-3p	抑制鼻咽癌细胞增殖	下调细胞周期相关蛋白表达	鼻咽癌	[22]
miR-BART12	抑制胃癌细胞增殖	未知	胃癌	[69]
miR-BART1-3p	抑制胃癌细胞增殖	下调酪氨酸激酶受体EphA2	胃癌	[71]
miR-BART18-5p			胃癌	[71]
miR-BART1-5p	抑制胃癌细胞增殖	靶向乙酰葡糖胺基转移酶GCNT3	胃癌	[72]
miR-BART4-3p	抑制胃癌细胞增殖	靶向与细胞增殖相关的基因AXL	胃癌	[73]
miR-BART4-5p	抑制胃癌细胞凋亡	下调BID表达	胃癌	[74]
	促进细胞迁移和侵袭	下调PTEN表达	鼻咽癌	[74]
miR-BART1-3p	抑制细胞凋亡	下调肿瘤抑制因子DAB2表达	胃癌	[75]
miR-BART5-3p	抑制肿瘤细胞凋亡	靶向PUMA上游转录因子TP53	胃癌	[77]
			鼻咽癌	[77]
miR-BART20-5p	抑制肿瘤细胞凋亡	下调BAD表达	胃癌	[67]
miR-BART1-3p	促进鼻咽癌转移	激活 PI3K-AKT, FAK-p130和SHC-MAPK通路	鼻咽癌	[85]
miR-BART1-5p				[85]
miR-BART9	促进鼻咽癌细胞迁移	抑制E-钙黏蛋白表达, 诱导EMT	鼻咽癌	[80]
miR-BART8-3p	促进鼻咽癌侵袭和转移	激活NF-κB和ERK1/2信号通路	鼻咽癌	[83]

(表1续)

非编码RNA	功能	机制	肿瘤类型	参考文献
miR-BART12	促进肿瘤细胞迁移和侵袭	下调TPPP1	鼻咽癌	[82]
miR-BART11	促进胃癌细胞迁徙	靶向FOXP1增加IL-1β, IL-6和IL-10的分泌	胃癌	[88]
EBER	促进凋亡抵抗	抑制PKR信号	淋巴瘤	[14,93,94]
		抗凋亡蛋白Bcl-2表达		[93]
	促进细胞转化和肿瘤发生	诱导细胞因子IL-6表达	淋巴瘤	[97]
		诱导B淋巴瘤细胞中的IL-10和T淋巴瘤细胞中的IL-9表达	淋巴瘤	[98]
	促进肿瘤发展	诱导IGF1表达	鼻咽癌	[99]
	促进血管生成	通过外泌体定位于血管内皮细胞, 促进血管生成	鼻咽癌	[100]

展[3,32~34].

EBV编码的另一个lncRNA位于BamHI A区域. BamHI A区域编码的mRNA通过可变剪接产生的转录本被称为BamHI A右向转录本(BART)[35]. BART是EBV相关上皮肿瘤中含量最高的病毒PolyA RNA, 包含多个ORF, 能编码四种lncRNA, 分别为BARF0, A73, BART和RPMS1[33,35].

在EBV感染的细胞中, BART lncRNA显著影响细胞黏附、氧化还原酶、鼻咽癌转移、炎症和免疫等相关基因表达[35~38]. 研究发现, 通过调节Pol II启动子区域, BART lncRNA似乎影响IFN-β1和CXCL8的表达[37]. 此外, Marquitz等人[33]发现, BART lncRNA可以下调肿瘤抑制因子RASA1, 提示BART lncRNA可能促进肿瘤发生. 根据Marquitz等人[33]研究, BART lncRNA也可能调控组蛋白活性或DNA甲基化表型, 促进肿瘤发生. 另有研究报道, 在EBV阳性胃癌中EBV通过甲基化CpG岛启动子, 导致特定基因沉默[39,40]. 在EBV相关鼻咽癌组织中, 锌指蛋白Aiolos参与表观遗传修饰, 其表达显著高于正常组织. 进一步研究发现, BART lncRNA促进Aiolos表达, 提示BART lncRNA可能通过上调Aiolos参与表观遗传修饰调控, 促进鼻咽癌发生发展[37,41,42].

BART lncRNA也参与调节未折叠蛋白反应(unfolded protein response, UPR)基因表达[33]. 研究显示, BART lncRNA可能直接或间接下调UPR主要转录因子(包括XBP1, ATF4和ATF6)[33], 这些转录因子下调可能是导致EBV相关肿瘤发生过程中应激反应基因减少的原因. 另有研究报道, 病毒致瘤蛋白LMP1通过NF-κB上调BART lncRNA水平[38]. 这些研究表明, 在EBV相关肿瘤中LMP1可能通过上调BART lncRNA减少UPR相关基因表达, 这与近期研究发现的鼻咽癌中LMP1能直接抑制某些UPR通路的结果相一致[43]. 综上, BART lncRNA可能通过调控多条信号通路促进病毒肿瘤发生, 但其调控相关基因表达的分子机制仍需进一步研究.

1.2 EBV circRNAs

circRNA是RNA将下游5′剪接供体反向剪接到上游3′剪接受体, 通过共价键产生的封闭环状RNA分子. circRNA可通过 (ⅰ) 海绵竞争性机制抑制miRNA靶向相应线性mRNA的能力, (ⅱ) 顺式转录调控相关基因, 或(ⅲ) 通过调节RNA剪接发挥其功能[44]. 研究表明, EBV可以编码EBNA1, RPMS1/BART, BHLF1和LMP2A的circRNA, 表达于不同的潜伏期[45]. 例如, EBV核抗原EBNA RNA剪接产生的circEBNA_U, 存在于 I期和III期潜伏感染的B细胞中, 而circBARTs在不同潜伏期均大量表达[46~48]. *BART*基因具有丰富的可变剪接位点, 可编码4种不同的circBART, 即circBART1.1 (711 nt,包括外显子II, IIIa, IIIb, IV和内含子IIIa)、circBART1.2 (509 nt, 包括外显子II, IIIa, IIb和IV)、cirBART2.1 (609 nt, 包含外显子IIIa, IIIb, IV和内含子IIIa)和circBART2.2 (399 nt, 包括外显子IIIa, III和IV)[25]. circLMP2和circBHLF1分别由*LMP2*和*BHLF1*基因的外显子反向剪接产生, 主要在感染EBV的增生性淋巴组织中表达[47].

目前, EBV circRNA作为miRNA海绵在鼻咽癌中已经得到证实. 例如, 在EBV相关鼻咽癌中, circRPMS1通过海绵作用竞争性抑制miR-31, miR-203和miR-451, 从而抑制EBV阳性鼻咽癌细胞增殖和EMT, 并且诱导细胞凋亡[49](表1). 此外, Qiao等人[48]在

感染EBV的HEK293细胞中提取circRNAs并分析其潜在的miRNA结合位点后, 确定了多达56种人类miRNA候选物. 其中, 丰度最高的前5个miRNA分别为miR-15b5p, miR-30c-1-3p, miR-30c-2-3p, miR-424-5p和miR4286, 并预测这些miRNA可调控多达1404个靶基因[48]. 这些基因富集于致癌和炎症通路, 提示EBV circRNAs可能与EBV相关肿瘤的发生密切相关.

胃癌中, EBV circRNA被发现可以通过调控相关基因转录促进胃癌发展. 研究表明, 在EBV相关胃癌中, EBV circRPMS1通过上调METTL3表达, 促进胃癌细胞增殖、迁移和侵袭, 并抑制其凋亡[25]. 机制上, EBV circRPMS1与Sam68结合, 促进其与METTL3启动子相互作用, 导致METTL3反式激活, 从而促进胃癌进展[25]. 此外, 在EBV相关的胃癌中, EBV circLMP2A的高表达与EBV相关胃癌患者的转移和不良预后显著相关[26]. 进一步研究显示, EBV circLMP2A通过靶向miR-3908/TRIM59/p53轴诱导和维持肿瘤干细胞干性[26](表1).

EBV circBART还被发现和鼻咽癌免疫逃逸有关(表1). Ge等人[27]发现, EBV编码的circBART2.2在鼻咽癌中高表达. circBART2.2通过与RIG-I的解旋酶结构域相互作用激活转录因子IRF3和NF-κB, 从而上调PD-L1并抑制T细胞功能, 导致肿瘤免疫逃逸. 该研究揭示了EBV circRNA通过与蛋白互作激活下游转录因子发挥功能的一种新模式[27]. 基于EBV circRNA在肿瘤增殖、存活、迁移、侵袭和免疫逃逸中扮演的重要角色, 靶向EBV circRNA可能是治疗EBV相关肿瘤的有效策略.

2 EBV编码的miRNA

miRNA是一类短链非编码RNA, 通过与靶mRNA的3′-非翻译区碱基互补配对, 促进靶mRNA降解. 研究表明, miRNA介导的基因调控与乳腺癌、肺癌、胃癌和肝癌等恶性肿瘤发生密切相关. EBV是第一种被发现编码miRNA的病毒[50]. EBV编码的miRNA主要来源于病毒基因组两个区域, 即BART和BHRF1区域[21,50]. BHRF1 miRNA在潜伏期III的淋巴细胞中高表达[18,51,52], 而BART miRNA在所有感染EBV的细胞系中都被发现, 包括鼻咽癌、胃癌和淋巴瘤[53~56]. 鉴于来自BART区域的miRNAs在不同肿瘤中被广泛发现, 而BHRF1 miRNA很少在EBV相关的胃癌和鼻咽癌中表达, 以下主要讨论BART miRNA在上皮细胞肿瘤中的表达及其相关机制.

*BART*基因包含两个由22个前体miRNA组成的簇(miR-BART1~22), 这两个簇主要位于内含子1中. 第一个簇包含8个miR-BART, 第二个簇包含13个miR-BART. miR-BART2位于两个簇外, 存在于*BART*基因的内含子4中[18,57]. 这些前体miRNA表达由*BART*启动子P1和P2控制. 前体miRNA表达后被RNase III酶Dicer切割, 从正向-5p链和反向-3p链中产生40个成熟的miRNA[17,50,57,58]. 研究表明, BART miRNA和广泛的肿瘤生物学行为有关, 包括肿瘤细胞增殖、存活、凋亡、侵袭和免疫监视等[59~62].

2.1 BART miRNA与肿瘤细胞增殖

肿瘤细胞增殖是EBV相关肿瘤的重要生物学特征. 不同的BART miRNA对细胞增殖的影响截然不同, 甚至相反. 根据对细胞增殖的影响, 来自两个簇的BART miRNAs大致可以分为两群, 一群促进肿瘤细胞增殖, 另一群抑制肿瘤细胞增殖.

促进肿瘤细胞增殖的BART miRNAs包括miR-BART13[63], miR-BART2-5p[64], miR-BART10-3p[65,66], miR-BART11-5p[64], miR-BART20-5p[67]和miR-BART4[68](表1). 鼻咽癌中, miR-BART13可以和NKIRAS2的3′-UTR结合, 下调其表达, 从而抑制NF-κB信号, 导致鼻咽癌细胞增殖活性下降[63]; 另一个miR-BART10-3p可以通过直接靶向ALK7通路促进鼻咽癌细胞增殖[65]. 在EBV相关的胃癌中, miR-BART2-5p和miR-BART11-5p通过直接靶向Rb和p21, 促进胃癌细胞增殖[64]. miR-BART10-3p则被发现可以通过结合DKK1 mRNA的3′-UTR下调其表达, 促进胃癌细胞增殖[66]. 另有研究发现, miR-BART20-5p可以和BAD mRNA的3′-UTR结合降低BAD表达, 促进EBV相关胃癌细胞增殖, 而miR-BART20-5p抑制剂则可逆转这一现象[67]. 此外, miR-BART4在EBV阳性鼻咽癌组织中高表达, 且与鼻咽癌中肿瘤抑制因子PTEN的低表达显著相关. 研究表明, miR-BART4的抑制可增加PTEN的mRNA和蛋白水平, 导致细胞增殖活性下降, 暗示PTEN可能是miR-BART4直接下游靶基因[68].

抑制肿瘤细胞增殖的BART miRNAs包括miR-BART4[68], miR-BART6-3p[22], miR-BART12[69], miR-

BART16[70], miR-BART1-3p[71], miR-BART1-5p[72], miR-BART4-3p[73]和miR-BART18-5p[71](表1). miR-BART6-3p是一个被鉴定为抑制鼻咽癌细胞增殖的BART miRNA. Wang等人[22]发现, miR-BART6-3p通过靶向lncRNA (LOC553103)增加STMN1 mRNA的不稳定性, 下调细胞周期相关蛋白表达, 从而抑制鼻咽癌细胞增殖. 在另一项研究中, miR-BART12被认为抑制胃癌细胞增殖, 但具体机制仍需要进一步阐明[69]. 同样在EBV阳性的胃癌细胞中, 与细胞增殖相关的乙酰葡糖胺基转移酶GCNT3呈低表达. 进一步研究表明miR-BART1-5p直接靶向GCNT3, 抑制胃癌细胞增殖[72]. 另有报道显示, 在EBV感染的胃癌细胞中, miR-BART4-3p直接靶向与细胞增殖相关的基因*AXL*, 从而抑制胃癌细胞增殖[73]. 此外, Shi等人[71]发现, miR-BART1-3p和miR-BART18-5p直接和酪氨酸激酶受体EphA2 mRNA的3′-UTR结合, 下调其表达, 抑制胃癌细胞增殖. 上述研究表明, BART miRNAs通过多种信号通路调控肿瘤细胞增殖, 构成了一个正/负调控网络, 进一步提示了EBV致瘤机制的复杂性.

2.2 BART miRNA与肿瘤细胞凋亡

来自两个BART簇的miRNA通过与细胞凋亡通路的特异性相互作用增强细胞凋亡抗性. 目前被鉴定为抑制肿瘤细胞凋亡的BART miRNA包括miR-BART4-5p[74], miR-BART1-3p[75], miR-BART5-5p[76], miR-BART5-3p[77], miR-BART16-5p[78], miR-BART2-5p[79], miR-BART11-5p[64], miR-BART4-3p[73]和miR-BART20-5p[67](表1). Shinozaki-Ushiku等人[74]发现, miR-BART4-5p直接和细胞凋亡基因*BID* mRNA的3′-UTR结合, 下调*BID*表达, 从而抑制EBV相关胃癌细胞凋亡. 近期研究发现, miR-BART1-3p可直接靶向肿瘤抑制因子DAB2 mRNA的3′-UTR, 抑制细胞凋亡, 同时增强肿瘤细胞的迁移能力[75]. 此外, miR-BART5-5p被发现在EBV感染的鼻咽癌和胃上皮细胞中高表达, 直接靶向促凋亡*PUMA* mRNA (*p53*上调的细胞凋亡基因), 促进肿瘤细胞存活[76]. 与此类似, miR-BART5-3p可以通过靶向*PUMA*上游转录因子TP53下调*PUMA*表达, 从而抑制肿瘤细胞凋亡[77]. 在胃癌AGS细胞系中, miR-BART20-5p通过直接结合细胞凋亡基因*BAD* mRNA的3-UTR, 下调*BAD*表达, 抑制AGS细胞凋亡[67]. 另有研究报道, 在EBV相关的胃癌中, miR-BART2-5p和miR-BART11-5p可以直接靶向肿瘤抑制因子RB和细胞增殖抑制基因*p21*, 从而抑制胃癌细胞凋亡, 但其分子机制仍需进一步研究[64].

2.3 BART miRNA与肿瘤侵袭和转移

侵袭和转移是导致肿瘤病人死亡的重要原因. 目前研究表明, BART miRNA通过靶向肿瘤侵袭转移相关通路参与EBV相关肿瘤侵袭和转移的各个生物学过程, 包括上皮-间质转化(epithelial-mesenchymal transition, EMT)和血管生成. 已经被鉴定为促进EBV相关肿瘤转移的BART miRNA包括miR-BART4[68], miR-BART4-5p[74], miR-BART9[80], miR-BART11[81], miR-BART10-3p[66], miR-BART12[69,82], miR-BART13[63], miR-BART8-3p[83], miR-BART18-5p[71], miR-BART1-5p[72,84], miR-BART1-3p[75,85], miR-BART2-5p[79,86]和miR-BART22[87]等.

鼻咽癌中, miR-BART4-5p直接结合肿瘤抑制因子*PTEN*, 导致*PTEN*的表达下调, 从而促进细胞迁移和侵袭[74](表1). 另有研究报道, miR-BART1的两个分支miR-BART1-3p和miR-BART1-5p通过直接结合*PTEN*, 并在体外激活PI3K-AKT, FAK-p130和SHC-MAPK通路, 促进鼻咽癌转移[85](表1). 此外, 研究表明, miRNA-BART2-5p在临床前鼻咽癌患者的血清中表达增加, 其拷贝数与疾病进展呈正相关. 进一步研究表明, miRNA-BART2-5p通过直接靶向Rho信号负调节因子RND3激活ROCK信号, 从而促进鼻咽癌转移[86]. 相似地, Wu等人[79]也发现miRNA-BART2-5p可以促进鼻咽癌转移. E-钙黏蛋白是一种膜蛋白, 在维持细胞-细胞连接和上皮表型方面起关键作用. 鼻咽癌细胞中, miR-BART9通过特异性抑制E-钙黏蛋白表达诱导肿瘤细胞EMT, 从而促进鼻咽癌细胞迁移[80]. 另有研究表明, miR-BART8-3p通过结合RNF38激活NF-κB和ERK1/2信号通路, 促进鼻咽癌迁移、侵袭和转移[83]. 微管蛋白聚合促进蛋白1 (TPPP1)参与微管动态组装, 重塑细胞形态, 促进EMT. Wu等人[82]发现, miR-BART12直接与TPPP1 mRNA的3′UTR区结合, 下调TPPP1, 从而促进EBV相关癌症(如鼻咽癌和胃癌)的侵袭和迁移. 在鼻咽癌细胞中, miR-BART10-3p可下调蛋白酶体(βTrCP) *BTRC*基因表达, 导致β-catenin和Snail表达上调, 从而促进肿瘤细胞迁移和侵袭[66].

胃癌中，Yoon等人[88]发现，miR-BART17-5p靶向DNA锌指结合蛋白KLF2，促进胃癌细胞迁移和锚定非依赖性生长[]. 另有研究表明，miR-BART11通过靶向FOXP1增加IL-1β，IL-6和IL-10的分泌，促进胃癌细胞的迁徙[81]. DKK1是一种参与胚胎发育的可溶性分泌蛋白，抑制Wnt信号通路. 已有研究发现，miR-BART10-3p通过靶向DKK1诱导EMT，从而促进EBV相关胃癌的迁移、侵袭和转移[66].

2.4 BART miRNA与肿瘤免疫监视

EBV可感染大多数人群，受感染的个体对病毒可产生强烈的免疫反应, 特别是细胞毒性CD8 T细胞, 但被感染的细胞和EBV很难被清除. 这表明EBV病毒具备某些机制，阻止CD8 T细胞有效清除EBV感染细胞. 研究表明, BART miRNA通过靶向IL-12, MHCII类和溶酶体蛋白酶抑制CD4 T细胞对感染B细胞的免疫反应[89]. 此外, BART miRNAs还可以通过直接靶向肽转运蛋白亚基TAP2并降低TAP1亚基水平、MHCI类分子和EBNA1的表达水平，从而抑制EBV特异性CD8 T细胞对感染B细胞的识别和杀伤[89]. 众多研究表明，EBV miRNA利用多种途径，使病毒不仅能够逃避CD4 T细胞的监视，还可以规避抗病毒CD8 T细胞的识别和杀伤. STAT3是PD-L1的上游转录因子，PIAS3通过抑制STAT3促进PD-L1的表达. Yoon等人[90]研究表明，miR-BART5-5p能直接靶向PIAS3并激活STAT3，上调PD-L1的表达，从而抑制CD8 T细胞的细胞毒性. FOXP1和PBRM1是PD-L1的转录抑制因子. 研究表明，miR-BART11和miR-BART17-3p通过直接靶向FOXP1和PBRM1，促进PD-L1表达，从而抑制CD8 T细胞对鼻咽癌细胞的杀伤[91]. 综上所述，EBV miRNAs能通过不同途径抑制宿主免疫反应，增强EBV逃避免疫监视的能力，从而削弱CD8 T细胞对感染细胞的识别和杀伤，提示EBV miRNA可作为肿瘤免疫治疗的潜在靶点.

3 EBV编码的小RNA

EBER1和EBER2具有54%的系列同源性. 两者被161个碱基对隔开，并在EBV图谱上从左向右转录. 两者都含RNA聚合酶III转录控制区，可以被其转录. 此外, EBER1和EBER2的二级结构表现出明显的相似性，包含许多短茎环结构[92]. 研究表明, 二级结构的维持对EBER功能至关重要(表1).

3.1 EBER与细胞凋亡

EBER可通过其抗凋亡活性促进EBV相关肿瘤的恶性表型, 如EBERs在EBV阴性的Akata细胞中表达促进细胞生长和细胞凋亡抵抗[93]. PKR是双链RNA依赖激酶，能被双链RNA和I型干扰素(IFNα/β)激活，其信号激活导致细胞凋亡，是抗病毒和促凋亡的重要激酶. 早期研究发现，EBER作为双链RNA可能通过抑制PKR信号抑制细胞凋亡[93]. Nanbo等人[14]发现, EBV阴性淋巴瘤细胞在IFNα刺激下进入凋亡，而EBV阳性淋巴瘤细胞在IFNα刺激下展示对凋亡的抵抗. 这一抗凋亡效应是由EBER与PKR互作所介导. Wong等人[94]用编码EBER的质粒转染永生化鼻咽上皮细胞NP69，用dsRNA刺激诱导凋亡，发现表达EBER的细胞对dsRNA诱导的凋亡展现出抗性，而对照组对dsRNA诱导的凋亡敏感. 进一步研究发现，EBER阳性细胞的PKR磷酸化水平显著降低, 暗示EBER通过下调PKR活性抑制dsRNA诱导的细胞凋亡. Komano等人[93]发现，淋巴瘤细胞Akata中EBER过表达导致抗凋亡蛋白Bcl-2表达增加，从而抑制细胞凋亡. 然而，Yoshizaki等人[95]在EB病毒阳性的鼻咽癌细胞株NPC-KT中表达更高的EBER, 却未见任何抗凋亡的能力. 这些结果提示, EBER的抗凋亡活性在淋系和上皮来源的肿瘤细胞中可能存在不同的机制.

3.2 EBER与细胞转化和肿瘤发生

多年来, 人们一直致力于探讨EBER潜在的致瘤机制，认为EBER有助于淋巴瘤细胞的恶性表型[96,97]. 研究发现，EBER缺失病毒的50%转化剂量是EBER阳性EBV的1/100，从而提示EBER对EBV介导的淋巴细胞转化是必需的[97]. Komano等人[93]早期发现，在Akata细胞中EBERs的表达促进细胞生长并且增强了细胞对凋亡的抵抗能力. 此外，他们还发现EBER的表达增加Akata细胞在软琼脂中的生长能力，表明EBER具有潜在的致瘤能力[93]. Kitagawa等人[98]在不同的淋巴细胞中发现，EBER可通过诱导B淋巴瘤细胞中的IL-10和T淋巴瘤细胞中的IL-9表达促进淋巴瘤细胞增殖. 另一项研究表明，EBER2可通过上调细胞因子IL-6促进B淋巴细胞的生长转化[97]. IGF1是鼻咽癌和胃癌细胞的

自分泌因子, EBV阳性的鼻咽癌和胃癌样本中IGF1表达远高于EBV阴性样本. 进一步研究表明, EBER通过诱导IGF1表达促进EBV相关肿瘤发展[99]. 此外, Cheng等人[100]发现, EBER能通过外泌体定位于血管内皮细胞, 促进血管生成, 从而促进鼻咽癌生长. 以上研究有力地支持了EBER可促进细胞转化和肿瘤恶性表型, 但该报道存在争议, 例如, Swaminathan等人[101]发现, EBER缺失既不影响EBV感染效率, 也不影响淋巴细胞的转化效率. 这些有争议的报道可能与研究中使用的细胞株和实验方法有关, 有必要进一步在同一模型中加以阐明.

4 总结与展望

已知EBV与多种淋巴和上皮来源肿瘤发生有关[102,103]. 其中, EBV非编码RNA在EBV复制周期和相关疾病发生(包括癌症)中发挥着重要作用, 是一个新兴且活跃的研究领域. 近来, 新技术的发展促进了EBV非编码RNA相关信号通路的解析, 为EBV致癌机制的研究提供了新思路, 也为发现新的EBV相关肿瘤生物标志物和治疗靶点开辟了新方向.

本文对EBV非编码RNA的研究现状进行了总结, 该领域仍有许多问题亟待解答. 我们重点提出以下问题, 希望能启发未来的研究方向.

(1) 除了已知的通过与RNA或蛋白质互作发挥功能的机制, EBV非编码RNA是否可以作为独立的功能分子? 潜在的独立功能包括RNA拼接、RNA切割、RNA连接、生物大分子修饰等.

(2) EBV非编码RNA与宿主非编码RNA之间的关系如何? 尽管EBV和宿主来源的非编码RNA均可以作用于宿主基因产物, 它们之间可能的合作/互作关系仍待阐明.

(3) 能否通过分析EBV非编码RNA预测病毒免疫状态? EBV感染和复制状态与病毒的免疫清除密切相关, 而EBV非编码RNA在感染不同阶段的表达图谱的建立有着非常重要的临床意义.

(4) EBV非编码RNA在肿瘤免疫逃逸及肿瘤免疫治疗反应的作用及机制如何? 尽管已有研究表明, EBV非编码RNA在肿瘤免疫中发挥作用, 但具体机制仍不清楚. 有证据表明, EBV阳性肿瘤预后良好, 但其免疫学机制未知, 值得深入探讨.

(5) EBV非编码RNA作为生物标志物和治疗靶点在EBV相关肿瘤中的转化潜力如何? 利用细胞非编码RNA组合预测疾病转归和治疗反应已有深厚积累, 部分已在临床转化应用. EBV病毒非编码RNA的不同组合能否成为特异性强的生物标志物应当在临床研究中加以验证. 近年来, 核酸药物技术的成熟也为靶向EBV非编码RNA提供可能.

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The function and mechanism of EBV non-coding RNA in cancers

HE Jiang[1], XIONG Wei[2], LI Xin[3] & SUN LunQuan[1,4]

1 Xiangya Cancer Center, Xiangya Hospital, Central South University, Key Laboratory of Molecular Radiation Oncology Hunan Province, Hunan international Science and Technology Cooperation Base of Precision Medicine for Cancer, Changsha 410008, China;
2 Cancer Research Institute, Central South University, Changsha 410078, China;
3 Shenzhen Hospital, Southern Medical University, Shenzhen 518000, China;
4 Institute of Geriatric Oncology, National Clinical Research Center for Geriatric Diseases (Xiangya Hospital), Changsha 410008, China

Epstein-Barr virus (EBV) is classified within the gamma herpesvirus subfamily and is prevalent in the human population, exhibiting a significant association with various malignancies, including nasopharyngeal carcinoma, gastric cancer, and lymphoma. Nevertheless, the molecular mechanisms that drive EBV-associated carcinogenesis remain inadequately understood. EBV non-coding RNAs include long non-coding RNAs (EBV lncRNAs), EBV-encoded small RNAs (EBERs), and EBV microRNAs (miRNAs). Epstein-Barr virus (EBV) non-coding RNAs significantly influence various biological processes, including viral replication, latency, tumor cell survival, proliferation, metastasis, and immune evasion by their interactions with potential target genes. This area of research has garnered considerable attention from the EBV research community in recent years. In this review, we provide a comprehensive summary of the functions and signaling pathways associated with EBV non-coding RNAs, while also discussing critical issues within the field. We aim to offer novel insights for the clinical application of targeting EBV non-coding RNAs.

EBV, **EBV non-coding RNA**, **EBV lncRNA**, **EBER**, **EBV miRNA**

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