疱疹病毒编码的miRNA与病毒潜伏

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摘要 MicroRNA(miRNA)是一类长度约为22核苷酸的非编码小RNA, 能够在转录后水平上对基因的表达进行调节, 进而影响细胞活动。病毒依赖宿主细胞进行复制。宿主可以通过免疫系统抵御病毒的入侵, 病毒会发展不同的策略用于逃避宿主的免疫。目前, 一些病毒已经被证明可以编码miRNA来重塑细胞环境。疱疹病毒是一类双链DNA病毒, 该类病毒能够在宿主体内保持长久的潜伏状态。疱疹病毒家族的很多成员都能够表达miRNA。越来越多实验结果表明, 疱疹病毒的miRNA对于调节病毒潜伏以及抑制宿主的抗病毒免疫反应方面发挥着重要的作用。该文讨论了疱疹病毒所编码的miRNA的产生过程和功能, 希望有助于了解疱疹病毒的潜伏感染以及病毒与宿主的相互作用。

关键词 疱疹病毒; miRNAs; 潜伏感染; 免疫

MiRNAs Encoded by Herpesviruses and Latent Infection

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Abstract MicroRNAs (miRNAs) are noncoding RNA molecules ~22 nucleotides in length that can modulate a range of fundamental cellular processes through post transcriptional gene regulation. Viruses depend on the host cellular machinery for their propagation and survival. The host can eliminate invading pathogens through the immune system. Conversely, viruses have developed various strategies to evade host immunity. Several viruses have been shown to use virally encoded miRNAs to reshape the cellular environment. Herpesviruses are a large family of double-stranded DNA viruses that can establish long-term, latent infections. MiRNAs are expressed by various members of the herpesvirus family. Increasing evidence suggests that viral miRNAs play a critical role in regulating viral latency, as well as in attenuating potentially inhibitory host antiviral immune responses. Here we discuss the expression and functions of miRNAs encoded by herpesviruses for further knowledge of latent infection and interactions between viruses and the host.

Keywords herpesviridae; miRNA; latent infection; immunity

疱疹病毒能够在宿主体内形成潜伏感染(latent infection)且往往潜伏终生, 疱疹病毒借助潜伏将其与宿主免疫系统隔离, 给抗病毒治疗造成很大的困难。研究发现, 疱疹病毒感染宿主时能编码产生miRNA。2003年, Pfeffer等[1]报道了疱疹病毒的miRNA的存在, 他们发现了爱泼斯坦–巴尔病毒(Epstein-Barr virus, EBV)在人类B淋巴细胞(B lymphocytes)中所表达的5种病毒miRNA。随后越来越多病毒编码的miRNA被人们发现[2]。目前所知EBV编码至少44种miRNA[3-5], 而与之相关的卡波

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济肉瘤相关疱疹病毒(Kaposi's sarcoma-associated herpesvirus, KSHV)编码了25种miRNA[2,6-7]。类似地, 人巨细胞病毒(human cytomegalovirus, HCMV)编码26种miRNA[2,8-9], I型和II型人类单纯疱疹病毒(herpes simplex virus type 1 and 2, HSV-1/2)分别编码至少27种和24种miRNA[10-11]。miRNA作为一种重要的调控分子参与了基因的转录后调控, 而迄今对疱疹病毒潜伏的机制未完全明确。本文综述了疱疹病毒编码的miRNA对病毒潜伏的调控作用, 希望有助于未来的疱疹病毒潜伏机制的研究。

1 疱疹病毒miRNA的产生与作用机制

1.1 疱疹病毒利用宿主的细胞完成miRNA的表达和进行miRNA介导的调控

首先, RNA聚合酶II转录成初始miRNA(pri-miRNAs), 该种RNA的长度由几百到上千个核苷酸不等, 类似于编码蛋白的转录本, 该种长链RNA有多腺苷酸尾巴和磷酸化加帽[12]。初始miRNA在细胞核被RNase III酶Drosha以及伴侣蛋白DGCR8 (microprocessor complex subunit 8, DGCR8)复合酶剪切形成具有发夹结构、长度为60~80核苷酸的RNA, 称为前体miRNA(pre-miRNAs)[13-14]。这些前体miRNA随后便由运输蛋白exportin-5运输至细胞质中, 然后, Dicer(RNA酶III)再经过进一步加工[15-17]。Dicer是一种剪切蛋白, 可以将pre-miRNAs加工成18~23碱基对的双链RNA, 通常情况下这种RNA双链之间是不完全互补的[15-17]。在RNA诱导的沉默复合体(RNA induced silencing complex, RISC)中, RNA双链与Ago蛋白(Argonaute)、Dicer酶和GW182蛋白相结合, RNA双链结构被解开[12,18-20]。随后一条链被降解, 另一条链(成熟的miRNA)被保留下来[21]。RISC由miRNA引导, 特异性识别和结合信使RNA(messenger RNA, mRNA), miRNA的目标位点通常位于mRNA的3′端非编码区(3′ end untranslation regions, 3UTRs)[22-23]。当miRNA与mRNA不完全互补时, miRNA通常会抑制mRNA的翻译; 当miRNA与mRNA序列完全互补时, miRNA通常会促进目标mRNA的降解[18]。当序列不完全互补时, miRNA种子序列(seed sequence, miRNA的第2~8位核苷酸序列)与mRNA的配对, 对于miRNA的靶位识别和功能来说至关重要[18,24-25]。miRNA种子序列与mRNA的完全互补能够抑制mRNA翻译, 但这种情况也不是绝对的[26]。

1.2 疱疹病毒miRNA的运输

疱疹病毒在借助宿主表达miRNA后, 能够通过细胞的胞外膜泡(extracellular vesicles), 完成miRNA在细胞间的传递。最新的研究发现, 在被EBV感染后, B淋巴细胞能够持续向外分泌包被病毒miRNA的胞外膜泡。胞外膜泡中的病毒miRNA借助胞吞作用(endocytosis)被运输至其他细胞, 包括单核细胞(monocytes)、单核细胞转化的树突状细胞(monocyte-derived dendritic cell, mDC)、浆细胞样树突状细胞(plasmacytoid dendritic cells)和上皮细胞(epithelial cells)[27-29]。但是关于胞外膜泡中携带的病毒miRNA的功能现在尚未有定论, 因为胞外膜泡中携带的miRNA的量非常低[30]。另外, 有研究发现, 具有感染力的病毒颗粒(infectious virus particles)也能够携带miRNA, 这些miRNA能够在病毒感染初期发挥调节作用[31]。

2 疱疹病毒的基因表达与潜伏

疱疹病毒复制过程中, 基因通过严格的级联调控被转录和表达, 根据时间先后顺序分为即早期基因(immediate-early gene, IE)、早期基因(early gene, E)、晚期基因(late gene, L)三类。即早期蛋白是病毒感染后最先表达的, 通常是调节蛋白, 疱疹病毒潜伏感染与裂解性感染(lytic infection)的相互转换与病毒即早期蛋白的表达密切相关[32]。早期基因的表达依赖于即早期蛋白的诱导, 早期基因的产物更多样化, 其通常参与病毒基因组复制或者调节宿主的免疫反应。晚期基因的产物主要是病毒的结构蛋白, 包括一些糖蛋白、衣壳蛋白和内膜蛋白等, 与病毒的吸附、进入和融合以及病毒的抗原性(antigenicity)有关[33-34]。

在疱疹病毒的潜伏过程中, 病毒能在宿主体内持续存在但宿主本身不表现任何相关的临床症状。潜伏感染病毒可由潜伏状态被再次激活并伴随具感染力的病毒颗粒产生。病毒处于潜伏感染状态时, 不表达任何相关的裂解性基因(lytic gene), 病毒蛋白质的表达受到普遍压制, 但会表达一些长非编码RNA(long non-coding RNAs, lncRNAs)[11]和miRNA, 同时在宿主体内可检测到病毒基因组的存在[35]。

疱疹病毒在宿主体内建立潜伏感染的原因主要有以下几个方面: (1)病毒感染后免疫逃逸(immune evasion); (2)病毒本身对即早期蛋白表达的

抑制以及调控; (3)病毒基因组与宿主细胞的相互作用, 包括病毒基因对细胞蛋白、mRNA合成的抑制、病毒mRNA的选择性翻译、疱疹病毒对细胞周期系统及细胞凋亡的调控作用等。

现有数据表明, 疱疹病毒的miRNA在潜伏期表现活跃。在KSHV中, 病毒miRNA以簇状的形式存在, 它们都来自于病毒在潜伏时转录出的一种初始miRNA前体[36]。在HSV-1中, 有6种病毒miRNA在潜伏期大量表达[37-40]。EBV的miRNA的表达可见于其复杂生命周期的各个时期[5]。HCMV在裂解感染期和潜伏期都表达miRNA[9]。

疱疹病毒表达miRNA十分活跃, 但是在非疱疹病毒的DNA病毒中, miRNA的表达却很是少见, 这是疱疹病毒区别于其他DNA病毒的一个重要特征, 这使人推测疱疹病毒miRNA的表达与病毒潜伏有关。一方面, miRNA可能通过调节病毒基因表达来调节病毒进入或离开潜伏[41]。其他DNA病毒不能建立潜伏, 细胞受到感染后会迅速裂解死亡, 因此在周期较短的裂解感染过程中, 作用于mRNA的miRNA没有时间发挥重要作用; 另一方面, 避免宿主免疫反应对于病毒潜伏的建立至关重要。miRNA不会成为激起宿主免疫反应的抗原, 因此疱疹病毒可以利用miRNA下调自身基因的表达, 降低病毒的抗原性; 或者靶向宿主基因, 下调宿主免疫, 达到免疫逃逸的目的。

3 疱疹病毒的miRNA限制自身基因的表达

疱疹病毒的miRNA通过靶向病毒的即早期基因的mRNA, 下调其基因的表达, 抑制病毒的生产性的裂解感染和阻止病毒的激活[8]。在潜伏期, HSV-1的miR-H6大量表达, 可以抑制HSV-1的即早期蛋白ICP4(infected cell protein 4)的表达, 有助于维持HSV-1的潜伏或阻止病毒的激活[38]。HCMV的病毒miRNA也被发现能够下调即早期蛋白的表达, HCMV的miR-ul112-1能够下调即早期蛋白IE72/IE1的表达[41-43], 阻止病毒进入裂解感染期。

疱疹病毒也能够通过miRNA的调节作用, 控制病毒基因产物的表达, 降低病毒的抗原性和细胞毒性, 减少由病毒抗原激起的细胞凋亡和由病毒毒性作用(toxic effect)造成的细胞死亡, 有助于病毒建立潜伏[44-45]。在EBV感染过程中, 病毒潜伏膜蛋白1(latent membrane protien 1, LMP1)的表达和EBV编码的核抗原蛋白1(epstein barr virus-encoded nuclear antigen1, EBNA1)均受到miR-BARTs的调控[46-47]。LMP1是EBV在潜伏期表达的一种细胞质信号分子, 其对细胞有双向调控作用: 低水平的LMP1能够诱导细胞生长和分化; 高水平的LMP1会导致细胞生长停滞和细胞凋亡[46,48]。病毒通过miRNA调节LMP1的表达, 减少由过量的LMP1表达引起的细胞凋亡。EBNA1是病毒基因组在细胞中维持和细胞分裂时病毒基因组复制所必需的[49]。然而, EBNA1会成为效应T细胞(T lymphocytes)的靶位, 病毒通过miRNA下调EBNA1的表达, 降低了EBV的免疫原性, 减弱效应T细胞的杀伤作用, 达到免疫逃逸[49]。HSV-2编码的miR-I, 能够抑制病毒蛋白ICP34.5(infected cell protein 34.5)的表达[50]。ICP34.5是一种毒性因子(toxic factor), 实验证明, *ICP34.5*基因缺失的HSV-1病毒具有更低的神经毒性(neurovirulence)[51]。在miR-I过表达细胞中, 病毒蛋白ICP34.5的表达被下调时, 病毒的裂解感染就会受到抑制, 表现出更低的神经毒性, 神经元在病毒感染后不会引起强烈的炎症反应, 这有利于病毒潜伏并与宿主长期共存。同样地, HSV-1也能通过编码miR-H2降低病毒的神经毒性, 维持宿主细胞的存活[52-53]。

4 疱疹病毒的miRNA靶向宿主免疫相关的基因

疱疹病毒miRNA能够靶向宿主固有免疫(innate immunity)或适应性免疫(adaptive immunity)相关的基因, 完成免疫逃逸, 促进和维持病毒潜伏。

固有免疫中的重要成员包括I型干扰素(type I interferon)和炎症小体(inflammasome)。I型干扰素能够激活STAT(signal transducer and activator of transcription)转录因子的表达, 从而诱导干扰素激活基因(IFN-stimulated genes, ISGs)在细胞以及其相邻细胞中的表达, 最终起到抗病毒感染的作用。宿主也以可通过炎症小体, 激活下游的信号通路, 诱导炎症因子分泌, 抵抗病原入侵及维持机体免疫系统稳定。现有数据表明, 有多种疱疹病毒的miRNA能够靶向I型干扰素信号通路相关的蛋白, 限制ISGs的抗病毒效应[54-56]。EBV编码的miRNA能够下调单核细胞的炎症小体蛋白NLRP3的表达, 抑制炎症小体的抗病毒效应[57]。

固有免疫防线中的另一个成员是自然杀伤细

胞(natural killer cell, NK cell), NK细胞能够识别细胞膜表面配体, 并且杀伤病态细胞。据报道, HCMV编码的miR-ul112-1能够靶向一种NK细胞的细胞膜表面配体MICB(MHC class I polypeptide-related sequence B), 该种配体在NK细胞杀死病毒感染细胞的过程中发挥了关键作用[58]。在过表达miR-ul112-1的细胞中, MICB的表达受到抑制并且细胞对NK细胞的杀伤作用具有抵抗性。另一方面, MICB的表达也受到HCMV病毒蛋白UL16的抑制, 这表明, HCMV所表达的UL16和miR-ul112-1可以协同作用, 使得受感染的细胞躲过人类免疫系统的NK细胞的杀伤[58]。如上文所述, miR-ul112-1也下调了HCMV病毒的即早期蛋白质IE72/IE1的表达, 这是第一种被发现的能够同时靶向病毒和宿主基因的病毒miRNA。在EBV、KSHV和HSV中也能够发现类似的机制, EBV和KSHV能够通过miRNA下调MICB的表达, 躲避NK细胞的杀伤作用[48,58]; HSV-1编码的miR-H8能够干扰糖基化磷脂酰肌醇锚定信号通路(glycosylphosphatidulinositol anchoring pathway), 下调NK细胞配体的表达, 干扰免疫监视[45]。

疱疹病毒的miRNA也能够靶向适应性免疫相关的细胞基因, 对宿主的适应性免疫进行调节。适应性免疫的抗原递呈是一个复杂的过程, 病毒的miRNA能够对其中的某个过程进行干扰, 阻止病毒多肽的递呈过程, 下调适应性免疫和维持病毒潜伏。EBV通过miRNA抑制TAP复合体(TAP complex)以及溶酶体酶(lysosomal enzymes)的表达, 进而影响病毒抗原的加工以及病毒多肽的递呈[47,49]。疱疹病毒的miRNA还能抑制细胞表面一些重要的抗原递呈相关蛋白, 例如主要组织相容性复合体(major histocompatibility complex, MHC)和共刺激分子(co-stimulatory molecules)。这些蛋白并不是病毒miRNA的直接靶蛋白, 但是这些细胞表面蛋白受到LMP1的调控, LMP1能够诱导这些蛋白的表达, 病毒的miRNA能够下调病毒LMP1蛋白的表达来降低病毒的免疫原性, 下调病毒抗原的递呈[47,49]。

细胞因子(cytokines)和趋化因子(chemokines)能够调节宿主的抗病毒炎症反应, 增强适应性免疫, 因此它们也有可能成为病毒miRNA的目标。EBV编码的多种miRNA可对干扰素诱导的T细胞α趋化因子(IFN-inducible T-cell attracting chemokine α, I-TAC/CXCL11)表达产生抑制, 而CXCL11有趋化活化的T细胞(activated T cells)、中性粒细胞(neutrophil)和单核细胞的功能[59]。至少有5种EBV的miRNA能够靶向白细胞介素IL-12p40, 下调B淋巴细胞IL-12和IL-23的分泌, 这两种白细胞介素都是IL-12家族的成员, 而白细胞介素IL-12的一个重要功能就是促使初始CD4 T细胞分化成抗病毒的辅助T细胞(helper T lymphocytes, Th1 cells)[47,60]。类似地, HCMV编码的多种miRNA能够协同作用, 下调细胞因子的分泌[61-62]。

宿主细胞的存活是疱疹病毒潜伏的必要条件, 疱疹病毒能通过miRNA调控细胞周期, 抑制细胞凋亡, 维持病毒潜伏。EBV的miR-BART能够下调PUMA(p53 upregulated modulator of apoptosis)的水平, 减少细胞凋亡。PUMA属于Bcl-2家族BH3-only亚家族, 参与各种刺激诱导的p53依赖途径和非依赖途径的细胞凋亡过程, 是凋亡的关键分子之一[26]。疱疹病毒miRNA也能够靶向*Bim*基因(Bcl-2 interacting mediator of cell death), 下调Bim蛋白的表达, 抑制Bim诱导的细胞凋亡[63]。KSHV的miRNA miR-K9能够靶向*Gadd45B*基因, 防止受感染细胞生长停滞和细胞凋亡。*Gadd45B*是一个受p53和BRCA1调节的生长阻滞和DNA损伤基因, 在抑制细胞转化和肿瘤恶性进展中扮演重要的角色[64]。

综上所述, 疱疹病毒能够利用自身编码的miRNA, 调节病毒与宿主基因表达, 达到促进病毒潜伏的目的, 总结归纳见图1。

5 疱疹病毒的miRNA的功能研究和应用

疱疹病毒既然进化出表达miRNA的能力, 应该会利用这些miRNA调控病毒生命周期中的重要生物过程。虽然许多疱疹病毒的miRNA已经被发现, 但是我们对其功能的研究依然不够深入。如果需要进一步明确疱疹病毒miRNA的功能, 我们需要通过同源重组技术(homologous recombination)、细菌人工染色体(bacterial artificial chromosome, BAC)技术和Rec E/T同源重组技术等, 构建miRNA单基因和基因簇缺失的病毒株[52,65]。通过动物实验分析miRNA与疱疹病毒致病性的关系, 筛选并鉴定潜在调控疱疹病毒致病或致瘤表型的miRNA, 为进一步阐明它们的分子调控机制奠定基础。

随着对疱疹病毒了解的不断增加, 人们将重新认识疱疹病毒miRNA在建立潜伏和逃避宿主的固有免疫或适应性免疫系统的功能。现阶段, 病毒的

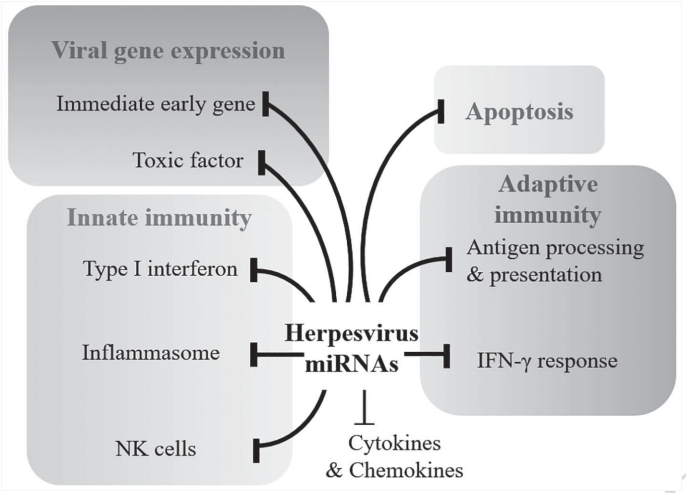

图1 疱疹病毒编码的miRNA的调节作用

Fig.1 Regulation of herpesvirus encoded miRNAs

miRNA已经被用作一种生物标志物(biomarkers), 应用于临床诊断。例如, HSV-2感染易诱发前列腺癌变, HSV-2编码的miRNA便可用于鉴定前列腺癌[66-67]。另外, EBV的miRNAs已经被用作为鼻咽癌病人的诊断和治疗的标志物[68]。最新的研究发现, miR-27的干扰作用能够下调HSV-1病毒的基因表达和降低病毒的复制, 这表明miRNA在疾病诊治方面的应用前景[45]。因此, 疱疹病毒治疗的新思路将从miRNA出发, 针对性地阻止或破坏疱疹病毒的感染。相信不久的将来, 随着越来越多的可靠数据的产生, miRNA作为疱疹病毒诊断、治疗、疗效预测及预后判断的重要分子标志将可能会得到极大程度的认可和推广。

参考文献 (References)

1 Pfeffer S, Zavolan M, Grasser FA, Chien M, Russo JJ, Ju J, *et al*. Identification of virus-encoded microRNAs. Science 2004; 304(5671): 734-6.

2 Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grasser FA, *et al*. Identification of microRNAs of the herpesvirus family. Nat Methods 2005; 2(4): 269-76.

3 Cai X, Schafer A, Lu S, Bilello JP, Desrosiers RC, Edwards R, *et al*. Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. PLoS Pathog 2006; 2(3): e23.

4 Grundhoff A, Sullivan CS, Ganem D. A combined computational and microarray-based approach identifies novel microRNAs encoded by human gamma-herpesviruses. RNA 2006; 12(5): 733-50.

5 Qiu J, Cosmopoulos K, Pegtel M, Hopmans E, Murray P, Middeldorp J, *et al*. A novel persistence associated EBV miRNA expression profile is disrupted in neoplasia. PLoS Pathog 2011; 7(8): e1002193.

6 Umbach JL, Cullen BR. In-depth analysis of Kaposi's sarcoma-associated herpesvirus microRNA expression provides insights into the mammalian microRNA-processing machinery. J Virol 2010; 84(2): 695-703.

7 Qin J, Li W, Gao SJ, Lu C. KSHV microRNAs: tricks of the devil. Trends Microbiol 2017; 25(8): 648-61.

8 Grey F, Antoniewicz A, Allen E, Saugstad J, McShea A, Carrington JC, *et al*. Identification and characterization of human cytomegalovirus-encoded microRNAs. J Virol 2005; 79(18): 12095-9.

9 Meshesha MK, Bentwich Z, Solomon SA, Avni YS. *In vivo* expression of human cytomegalovirus (HCMV) microRNAs during latency. Gene 2016; 575(1): 101-7.

10 Jurak I, Kramer MF, Mellor JC, van Lint AL, Roth FP, Knipe DM, *et al*. Numerous conserved and divergent microRNAs expressed by herpes simplex viruses 1 and 2. J Virol 2010; 84(9): 4659-72.

11 Phelan D, Barrozo ER, Bloom DC. HSV1 latent transcription and non-coding RNA: a critical retrospective. J Neuroimmunol 2017; 308: 65-101.

12 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116(2): 281-97.

13 Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, *et al*. The Microprocessor complex mediates the genesis of microRNAs. Nature 2004; 432(7014): 235-40.

14 Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, *et al*. The nuclear RNase III Drosha initiates microRNA processing. Nature 2003; 425(6956): 415-9.

15 Elbashir SM, Lendeckel W, Tuschl T. RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes Dev 2001; 15(2): 188-200.

16 Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 2001; 293(5531): 834-8.

17 Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science 2001; 294(5543): 858-62.

18 Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136(2): 215-33.

19 Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, *et al*. Argonaute2 is the catalytic engine of mammalian RNAi. Science 2004; 305(5689): 1437-41.

20 Liu J, Rivas FV, Wohlschlegel J, Yates JR, 3rd, Parker R, Hannon GJ. A role for the P-body component GW182 in microRNA function. Nat Cell Biol 2005; 7(12): 1261-6.

21 Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD. Asymmetry in the assembly of the RNAi enzyme complex. Cell 2003; 115(2): 199-208.

22 Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009; 19(1): 92-105.

23 Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell 2003; 115(7): 787-98.

24 Brennecke J, Stark A, Russell RB, Cohen SM. Principles of microRNA-target recognition. PLoS Biol 2005; 3(3): e85.

25 Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol Cell 2007; 27(1): 91-105.

26 Choy EY, Siu KL, Kok KH, Lung RW, Tsang CM, To KF, *et al*. An Epstein-Barr virus-encoded microRNA targets PUMA to promote host cell survival. J Exp Med 2008; 205(11): 2551-60.

27 Gallo A, Vella S, Miele M, Timoneri F, Di Bella M, Bosi S, *et al*. Global profiling of viral and cellular non-coding RNAs in Epstein-Barr virus-induced lymphoblastoid cell lines and released exosome cargos. Cancer Lett 2017; 388: 334-43.

28 Baglio SR, van Eijndhoven MA, Koppers-Lalic D, Berenguer J, Lougheed SM, Gibbs S, *et al*. Sensing of latent EBV infection through exosomal transfer of 5'pppRNA. Proc Natl Acad Sci USA 2016; 113(5): E587-96.

29 Nanbo A, Kawanishi E, Yoshida R, Yoshiyama H. Exosomes derived from Epstein-Barr virus-infected cells are internalized via caveola-dependent endocytosis and promote phenotypic modulation in target cells. J Virol 2013; 87(18): 10334-47.

30 Chevillet JR, Kang Q, Ruf IK, Briggs HA, Vojtech LN, Hughes SM, *et al*. Quantitative and stoichiometric analysis of the microRNA content of exosomes. Proc Natl Acad Sci USA 2014; 111(41): 14888-93.

31 Jochum S, Ruiss R, Moosmann A, Hammerschmidt W, Zeidler R. RNAs in Epstein-Barr virions control early steps of infection. Proc Natl Acad Sci USA 2012; 109(21): E1396-404.

32 Vere Hodge RA, Field HJ. Antiviral agents for herpes simplex virus. Adv Pharmacol 2013; 67: 1-38.

33 Johnston C, Koelle DM, Wald A. HSV-2: in pursuit of a vaccine. J Clin Invest 2011; 121(12): 4600-9.

34 Awasthi S, Friedman HM. Status of prophylactic and therapeutic genital herpes vaccines. Curr Opin Virol 2014; 6: 6-12.

35 Kesari S, Lee VM, Brown SM, Trojanowski JQ, Fraser NW. Selective vulnerability of mouse CNS neurons to latent infection with a neuroattenuated herpes simplex virus-1. J Neurosci 1996; 16(18): 5644-53.

36 Gottwein E, Cai X, Cullen BR. Expression and function of microRNAs encoded by Kaposi's sarcoma-associated herpesvirus. Cold Spring Harb Symp Quant Biol 2006; 71: 357-64.

37 Cui C, Griffiths A, Li G, Silva LM, Kramer MF, Gaasterland T, *et al*. Prediction and identification of herpes simplex virus 1-encoded microRNAs. J Virol 2006; 80(11): 5499-508.

38 Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Nature 2008; 454(7205): 780-3.

39 Du T, Han Z, Zhou G, Roizman B. Patterns of accumulation of miRNAs encoded by herpes simplex virus during productive infection, latency, and on reactivation. Proc Natl Acad Sci USA 2015; 112(1): E49-55.

40 Kramer MF, Jurak I, Pesola JM, Boissel S, Knipe DM, Coen DM. Herpes simplex virus 1 microRNAs expressed abundantly during latent infection are not essential for latency in mouse trigeminal ganglia. Virology 2011; 417(2): 239-47.

41 Murphy E, Vanicek J, Robins H, Shenk T, Levine AJ. Suppression of immediate-early viral gene expression by herpesvirus-coded microRNAs: implications for latency. Proc Natl Acad Sci USA 2008; 105(14): 5453-8.

42 Grey F, Meyers H, White EA, Spector DH, Nelson J. A human cytomegalovirus-encoded microRNA regulates expression of multiple viral genes involved in replication. PLoS Pathog 2007; 3(11): e163.

43 Lau B, Poole E, Van Damme E, Bunkens L, Sowash M, King H, *et al*. Human cytomegalovirus miR-UL112-1 promotes the down-regulation of viral immediate early-gene expression during latency to prevent T-cell recognition of latently infected cells. J Gen Virol 2016; 97(9): 2387-98.

44 Ressing ME, van Gent M, Gram AM, Hooykaas MJ, Piersma SJ, Wiertz EJ. Immune Evasion by Epstein-Barr virus. Curr Top Microbiol Immunol 2015; 391: 355-81.

45 Enk J, Levi A, Weisblum Y, Yamin R, Charpak-Amikam Y, Wolf DG, *et al*. HSV1 microRNA modulation of GPI anchoring and downstream immune evasion. Cell Rep 2016; 17(4): 949-56.

46 Lo AK, To KF, Lo KW, Lung RW, Hui JW, Liao G, *et al*. Modulation of LMP1 protein expression by EBV-encoded microRNAs. Proc Natl Acad Sci USA 2007; 104(41): 16164-9.

47 Tagawa T, Albanese M, Bouvet M, Moosmann A, Mautner J, Heissmeyer V, *et al*. Epstein-Barr viral miRNAs inhibit antiviral $CD4^+$ T cell responses targeting IL-12 and peptide processing. J Exp Med 2016; 213(10): 2065-80.

48 Riley KJ, Rabinowitz GS, Yario TA, Luna JM, Darnell RB, Steitz JA. EBV and human microRNAs co-target oncogenic and apoptotic viral and human genes during latency. EMBO J 2012; 31(9): 2207-21.

49 Albanese M, Tagawa T, Bouvet M, Maliqi L, Lutter D, Hoser J, *et al*. Epstein-Barr virus microRNAs reduce immune surveillance by virus-specific $CD8^+$ T cells. Proc Natl Acad Sci USA 2016; 113(42): E6467-e75.

50 Tang S, Bertke AS, Patel A, Wang K, Cohen JI, Krause PR. An acutely and latently expressed herpes simplex virus 2 viral microRNA inhibits expression of ICP34.5, a viral neurovirulence factor. Proc Natl Acad Sci USA 2008; 105(31): 10931-6.

51 Orvedahl A, Alexander D, Talloczy Z, Sun Q, Wei Y, Zhang W, *et al*. HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. Cell Host Microbe 2007; 1(1): 23-

35.

52 Jiang X, Brown D, Osorio N, Hsiang C, Li L, Chan L, *et al*. A herpes simplex virus type 1 mutant disrupted for microRNA H2 with increased neurovirulence and rate of reactivation. J Neurovirol 2015; 21(2): 199-209.

53 Jiang X, Brown D, Osorio N, Hsiang C, BenMohamed L, Wechsler SL. Increased neurovirulence and reactivation of the herpes simplex virus type 1 latency-associated transcript (LAT)-negative mutant dLAT2903 with a disrupted LAT miR-H2. J Neurovirol 2016; 22(1): 38-49.

54 Hooykaas MJG, van Gent M, Soppe JA, Kruse E, Boer IGJ, van Leenen D, *et al*. EBV MicroRNA BART16 suppresses type I IFN signaling. J Immunol 2017; 198(10): 4062-73.

55 Ramalingam D, Ziegelbauer JM. Viral microRNAs target a gene network, inhibit STAT activation, and suppress interferon responses. Sci Rep 2017; 7: 40813.

56 Lu Y, Qin Z, Wang J, Zheng X, Lu J, Zhang X, *et al*. Epstein-Barr virus miR-BART6-3p inhibits the RIG-I pathway. J Innate Immun 2017; 9(6): 574-86.

57 Haneklaus M, Gerlic M, Kurowska-Stolarska M, Rainey AA, Pich D, McInnes IB, *et al*. Cutting edge: miR-223 and EBV miR-BART15 regulate the NLRP3 inflammasome and IL-1beta production. J Immunol 2012; 189(8): 3795-9.

58 Stern-Ginossar N, Elefant N, Zimmermann A, Wolf DG, Saleh N, Biton M, *et al*. Host immune system gene targeting by a viral miRNA. Science 2007; 317(5836): 376-81.

59 Xia T, O'Hara A, Araujo I, Barreto J, Carvalho E, Sapucaia JB, *et al*. EBV microRNAs in primary lymphomas and targeting of CXCL-11 by ebv-mir-BHRF1-3. Cancer Res 2008; 68(5): 1436-42.

60 Song Y, Li X, Zeng Z, Li Q, Gong Z, Liao Q, *et al*. Epstein-Barr virus encoded miR-BART11 promotes inflammation-induced carcinogenesis by targeting FOXP1. Oncotarget 2016; 7(24): 36783-99.

61 Hook LM, Grey F, Grabski R, Tirabassi R, Doyle T, Hancock M, *et al*. Cytomegalovirus miRNAs target secretory pathway genes to facilitate formation of the virion assembly compartment and reduce cytokine secretion. Cell Host Microbe 2014; 15(3): 363-73.

62 Lau B, Poole E, Krishna B, Sellart I, Wills MR, Murphy E, *et al*. The expression of human cytomegalovirus microRNA miR-UL148D during latent infection in primary myeloid cells inhibits activin a-triggered secretion of IL-6. Sci Rep 2016; 6: 31205.

63 Marquitz AR, Mathur A, Nam CS, Raab-Traub N. The Epstein-Barr virus BART microRNAs target the pro-apoptotic protein Bim. Virology 2011; 412(2): 392-400.

64 Liu X, Happel C, Ziegelbauer JM. Kaposi's sarcoma-associated herpesvirus microRNAs target GADD45B to protect infected cells from cell cycle arrest and apoptosis. J Virol 2017; doi: 10.1128/JVI.02045-16.

65 Pan D, Pesola JM, Li G, McCarron S, Coen DM. Mutations inactivating herpes simplex virus 1 microRNA miR-H2 do not detectably increase ICP0 gene expression in infected cultured cells or mouse trigeminal ganglia. J Virol 2017; doi: 10.1128/JVI.02001-16.

66 Yun SJ, Jeong P, Kang HW, Kim YH, Kim EA, Yan C, *et al*. Urinary microRNAs of prostate cancer: virus-encoded hsv1-miRH18 and hsv2-miR-H9-5p could be valuable diagnostic markers. Int Neurourol J 2015; 19(2): 74-84.

67 Yun SJ, Jeong P, Kang HW, Shinn HK, Kim YH, Yan C, *et al*. Increased expression of herpes virus-encoded hsv1-miR-H18 and hsv2-miR-H9-5p in cancer-containing prostate tissue compared to that in benign prostate hyperplasia tissue. Int Neurourol J 2016; 20(2): 122-30.

68 Zhang G, Zong J, Lin S, Verhoeven RJ, Tong S, Chen Y, *et al*. Circulating Epstein-Barr virus microRNAs miR-BART7 and miR-BART13 as biomarkers for nasopharyngeal carcinoma diagnosis and treatment. Int J Cancer 2015; 136(5): E301-12.