INTRODUCTION

MicroRNAs (miRNAs) are the most studied and best characterized molecules in the class of small regulatory non-coding RNAs (Bartel, 2009). They are involved in several important biological processes and functions through post-transcriptional regulation of the expression of messenger RNAs (mRNAs), and their dysregulation is often cause or consequence of a variety of diseases, such as cancer and neurodegenerative disorders (Croce, 2009; Eacker et al., 2009). Cellular miRNAs can be packaged into different carriers and exported to recipient cells or released in small vesicles during apoptosis (Boon and Vickers, 2013; Hilton and Karpe, 2013). The discovery of extracellular miRNAs in biological fluids has started a new exciting field of research. Circulating miRNAs are small non-coding RNAs responsible of post-transcriptional regulation of gene expression through interaction with messenger RNAs (miRNAs). They are involved in important biological processes and are often dysregulated in a variety of diseases, including cancer and infections. Viruses also encode their own sets of miRNAs, which they use to control the expression of either the host’s genes and/or their own. In the past few years evidence of the presence of cellular miRNAs in extracellular human body fluids such as serum, plasma, saliva, and urine has accumulated. They have been found either cofractionate with the Argonaute2 protein or in membrane-bound vesicles such as exosomes. Although little is known about the role of circulating miRNAs, it has been demonstrated that miRNAs secreted by virus-infected cells are transferred to and act in uninfected recipient cells. In this work we summarize the current knowledge on viral circulating miRNAs and provide a few examples of computational prediction of their function.

Keywords: microRNA, viruses, exosomes, circulating microRNA, vesicles, body fluids

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EXTRACELLULAR CIRCULATING VIRAL miRNAs: CURRENT KNOWLEDGE AND PERSPECTIVES

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Viruses encode miRNAs. RNA interference (RNAi) most probably was originally selected as a primary mechanism of defense against harmful genetic elements be selectively packaged into different kinds of carriers, such as membrane-derived vesicles, lipoproteins, and ribonucleoprotein complexes, which protect them from degradation and export them to recipient cells where they exert their regulatory functions. Particularly, exosomes and microparticles (MPs) are two distinct classes of small membrane-enclosed vesicles released from cells, differing in size, biogenesis, and secretory mechanisms (Boon and Vickers, 2013). Exosomes are produced by the inward budding of the limiting membrane of multivesicular bodies (MVBs). They are smaller than MPs, which are instead formed by the outward budding and blebbing of the plasma membrane. Small sealed membrane vesicles that are produced from cells during apoptosis, called apoptotic bodies, can also be selectively packaged into different kinds of carriers, such as membrane-derived vesicles (Arroyo et al., 2011; Turchinovich et al., 2011; Vickers and Remaley, 2012; Rayner and Hennessey, 2013). Viral surface antigen particles may also carry specific miRNAs, as in the case of hepatitis B surface antigen particles which contain hepatocellular miRNAs bound to AGO2 (Novellino et al., 2012).

MicroRNA profiles of extracellular carriers show distinct sets of miRNAs than their parent cell-type, thus suggesting that some miRNAs might be transcribed only to be exported and not retained in the parent cell (Ohshima et al., 2010; Pigati et al., 2010). Selective packaging of miRNAs into vesicles is probably related to the specific biological functions of the secreted miRNAs.

Circulating miRNAs are highly stable and consistent among individuals of the same species. Specific miRNA expression signatures in extracellular environment have been identified in a variety of human diseases, including cancer and neurological diseases, revealing the diagnostic potential of circulating miRNAs as useful non-invasive biomarkers (Alexandrov et al., 2012; Fayyad-Kazan et al., 2013; Zeng et al., 2013).

Several in vitro studies have shown that miRNAs transferred by the different types of carriers are functional and can regulate gene expression in recipient cells. Apoptotic bodies generated from endothelial cells during atherosclerosis were shown to contain miR-126, which controls endothelial cell signaling in vitro and provides atherosprotective effects in vivo (Zettlcke et al., 2009).

Another study showed that endothelial cells can transfer functional miR-143 and miR-145 to smooth muscle cells where they mediate the reduction of atherosclerotic lesion formation in vivo (Horgenmüller et al., 2012). Similarly, circulating miR-150 is released by monocytes and taken up by endothelial cells where it regulates endothelial cell migration (Zhang et al., 2010).

Although the complete mechanism of gene regulation mediated by specifically selected circulating extracellular miRNAs has yet to be clearly demonstrated in vivo, these studies suggest a plausible form of cell-to-cell communication in which donor cells send their miRNAs to distant recipient cells where they exert their regulatory functions.
such as viruses. It is of relevant interest that in the evolutionary selection this mechanism was in turn exploited by viruses to their advantage while, as suggested by tenOever (2013), chordate use of small RNAs might exclusively have shifted to the silencing of genome-encoded transcripts and would at least not pose direct threat to RNA viral genome. The first report of viral-encoded miRNAs was published by Pfeffer et al. (2004) describing the cloning of viral miRNAs from cells infected with EBV. Among DNA viruses, which account for the majority of known virus-encoded miRNAs, 95% of viral miRNAs known today are of herpesvirus origin. The majority of natural viruses found to encode miRNAs have thus a DNA component to their replication cycle, can exploit the initiating host miRNA biogenesis machinery in the nucleus where they replicate, and cause long-term persistent infections. DNA viruses such as the ones belonging to the Herpesvirus, Polyomavirinae, Ascorvirus, Baculovirus, Iridovirus, and Adenovirinae families clearly match these characteristics (Sullivan et al., 2005; Gottwein et al., 2007; Choy et al., 2008; Hussain et al., 2008; Seo et al., 2009; Seto et al., 2010; Bauman et al., 2011; Marquitz et al., 2011; Suffert et al., 2011; Zhao et al., 2011; Lee et al., 2012) along with at least one member of the retrovirus family, bovine leukemia virus (BLV), which clearly encodes numerous miRNAs (Kincaid et al., 2012).

Despite the established case of BLV, viruses possessing positive or negative sense RNA or double-stranded RNA (dsRNA) genome are not widely accepted to naturally express miRNAs. Nevertheless, HIV-1 has been proven to encode two miRNAs and potentially a third. In fact, hiv1-mir-H1 has been proven to be responsible for inducing apoptosis and repressing host gene transcription (Koziol et al., 2009), while hiv1-mir-NS367 has been suggested as functional ortholog of hsa-miR192 (You et al., 2012). Finally, some evidence is present that the HIV-1 TAR element could be a potential viral miRNA (Houzet and Jeang, 2011), also considering its capability to target pro-apoptotic genes (Klasi et al., 2009).

All viral miRNAs can essentially be grouped into two classes: host analogs and virus-specific. Generally, though, their functions include prolonging latency of infected cells, evading the immune response, and regulating host or viral genes to limit the lytic cycle. Interestingly, all these functions are essential for infections to be persistent.

In fact, miRNAs are likely invisible to the adaptive immune system—a valuable trait for viruses that undergo persistent infection (Cullen, 2006). Thus, in viruses that establish a long-lasting latent infection, such as herpesviruses, one important benefit they could gain from employing miRNAs is the ability to regulate host and/or viral gene expression without having to elicit an antigenic immune reaction or directly suppressing components of the host immune system (Sullivan, 2008).

Preventing cell death seems an obvious advantage to viruses that cause persistent or latent infections. Several different viruses including Kaposi’s sarcoma-associated herpesvirus (KSHV), EBV, and Marek’s Disease Virus type 1 (MDV1) encode miRNAs that can play a subtle role in preventing apoptosis by targeting pro-apoptotic host genes and are also associated with tumorigenesis. PERSPECTIVES

VIRUSES CAN USE VESICLES TO EXPORT THEIR FUNCTIONAL miRNAs

Pegtel et al. (2010) were the first ones (and, to our knowledge, the only ones together with Meckes et al., 2010) to have demonstrated that virus-infected cells package virus-encoded RNAs, and specifically viral miRNAs, into exosomes which are exported into the extracellular space and eventually delivered to recipient, non-infected cells, favoring the repression of specifically important miRNA targets. EBV is a clear example of a virus that utilizes the exosome pathway for the selective secretion of viral and cellular proteins and miRNAs that likely participate in cell-to-cell communication in the absence of virus production, potentially modulating cell function.

As confirming proof, Pegtel et al. (2010) reported that EBV-infected activated B cells secrete exosomes containing viral miRNAs shown to be delivered and actively internalized by monocyte-derived dendritic cells in co-culture. In particular, the copy number of EBV-miRNA BART1-5p was consistently higher than other EBV-miRNAs and its level increased fourfold after additional 24 h co-culture. This resulted in a dose-dependent, miRNA-mediated repression of confirmed EBV target genes. More specifically, the viral miRNA BHRF1-3 was shown to suppress the expression of the immunostimulatory gene CXCL11 [Chemokine (C-X-C motif) ligand 11] and this repression was proven to be dependent on the amount of exosomes carrying the miRNA and was not recipient cell-type-specific. In addition, expression of EBV-miRNAs in EBV-infected circulating B cells was also investigated. The data collected suggested that in asymptomatic patients BART miRNAs are expressed by latently infected circulating B cells as well as present in non-infected non-B cells, supporting the possibility of miRNA transfer in vivo. This further supported the proposal that exosomes could most likely serve as deliverers of small RNA due to their specialized biogenesis and presumed entry route (Zomer et al., 2010). Later evidence showed that EBV-encoded miRNAs have been detected in exosomes from EBV-infected NPC cells, together with the LMP1 protein and other signal transduction molecules (Meckes et al., 2010), in accordance to other studies proving the presence of cellular miRNAs in tumor-derived exosomes (Taylor and Gercel-Taylor, 2008; Kharzaiha et al., 2012; Palma et al., 2012). Furthermore, differences detected in the levels of intracellular and exosomal miRNAs, in addition to differences even in the amount of enrichment between the individual exosomal miRNAs, suggest that some viral miRNAs might be specifically intended and selected to be packaged into exosomes and exert their functions in cells other than those producing them (Klihi et al., 2009; Meckes et al., 2010; Pegtel et al., 2010). Moreover, exosomes may also deliver cellular components of the RNA-induced silencing complex (RISC) to enhance viral miRNA function (Gibbons et al., 2009).

These results were greatly motivated by the assumption that exosomal exportation of miRNAs in general may have a fundamental role in intercellular communication despite the lack of concrete evidence (Valadi et al., 2007; Skog et al., 2008; Théry et al., 2009). Although functional significance of all these phenomena requires further investigation, these results suggest that a cellular
miRNA-loading mechanism may exist to direct specific miRNAs into intraluminal vesicles of multivesicular endosomes (MVEs) which could explain why exogenous extracellular miRNAs are capable of repressing targets in recipient cells at new subcellular compartments such as late endosomes (Morelli et al., 2004; Stern-Ginossar et al., 2007; Gibbings et al., 2009). Figure 1 depicts all the potential ways in which viruses could exploit extracellular particles to convey their miRNAs to non-infected recipient cells.

**FUNCTIONAL ANALYSIS OF CIRCULATING VIRAL miRNAs**

The correct identification of targets is fundamental to determine miRNA function. Computational miRNA target prediction is still a big challenge, mostly due to the fact that our knowledge about the mechanisms and the molecular rules of miRNA target recognition is still incomplete (Bartel, 2009). Nevertheless, there are many computational tools available online, which allow to identify the most probable miRNA targets and to uncover non-trivial relationships between miRNAs and other molecular actors (Cascione et al., 2013). These tools collect and integrate heterogeneous miRNA-related data retrieved from different sources, such as target prediction tools and expression profiles of miRNAs and mRNAs, in order to infer miRNA functions and produce general models of miRNA-mediated regulation in the context of complex processes. Few tools are available specifically for the analysis of viral miRNAs and they are limited to the prediction of new miRNAs and targets. RepTar and vHoT are databases of predicted interspecies interactions between viral miRNA and host genomes, while ViTa is a database containing predictions of host miRNA targets on viruses (Hsu et al., 2007; Elefant et al., 2011; Kim et al., 2012). miRiam is a software that has been used to predict potential human targets for viral miRNAs (Laganà et al., 2010). Finally, VMir and Vir-Mir are tools for the prediction of novel virus-encoded miRNAs (Li et al., 2008; Grundhoff, 2011). In regard to functional analysis, despite the lack of specific programs for viral miRNAs, general miRNA

**FIGURE 1** | Summary model of plausible mechanisms for export and functional delivery of viral miRNAs. The image depicts the possible means of transcription, packaging, and functional delivery of viral miRNAs during an infection. Virus-encoded miRNAs are transcribed by the infected cell (A). They could exploit various channels to reach extracellular space and, eventually, be delivered to recipient non-infected cells: inside apoptotic bodies after cell death (B), packaged into exosomes (C), or HDL/LDL molecules or even bound to AGO2 (D). Viral miRNAs may be uptaken by non-infected cells where they could exert their regulatory functions (E).
Table 1 | Functional enrichment analysis of circulating EBV miRNAs’ predicted targets.

| P-Value | Selected canonical pathways | Selected molecular and cellular functions | Selected diseases and disorders: cancer |
|---------|----------------------------|-------------------------------------------|---------------------------------------|
|         | Molecular mechanisms of cancer | Cell morphology                           | Leukemias and lymphomas                |
|         | PPARα/PPARγ activation       | Cell death and survival                    | Mesenchymal tumor                     |
|         | Wntβ-catenin signaling       | Cell cycle                                 | Mesenchymal tumor                     |
|         | p53 signaling                |                                           | Cell death and survival                |
|         | IL-6 signaling               |                                           | Cell cycle                             |
|         |                                |                                           |                                       |

The table summarizes the most relevant results, particularly associated to EBV-encoding miRNAs. The authors performed a functional enrichment analysis by comparing each gene target set with an annotated functional gene set corresponding to KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways and Gene Ontology biological processes. As further proof of principle, we used miRami in the prediction of the potential targets of EBV miRNAs in which exosomes are particularly enriched, as reported by Pegtel et al. (2010) (miR-BHRF1-1/-2-3p and miR-BARTI-3p/5p/-2-3p). Then, we used the tool Ingenuity pathway analysis (IPA) to perform a functional enrichment analysis of the predicted targets (http://www.ingenuity.com). The results show that subsets of the targets are significantly involved in cancer pathways, in particular leptomeningitis, and mesenchymal tumors, for which a connection with EBV had already been described (Cheruk et al., 2002; Monforte-Muñoz et al., 2003; Deyrup et al., 2006; Sunde et al., 2010). Other significant pathways include WNT/β-catenin signaling, interleukin 8 (IL-8) signaling, and P53 pathway (P < 0.0001), also previously described as related to EBV infections (Morrison et al., 2003; Everly et al., 2004; Ren et al., 2004; Webb et al., 2008; Forte and Luftig, 2009; Husaini et al., 2011; QingLing et al., 2011). The predicted targets are also enriched in GO terms such as cell death and survival and cell cycle (P < 0.04). Furthermore, although the significance of the P-Value is borderline (P = 0.4), it is worth to mention that the top tox functions reported by IPA include increased levels of alkaline phosphatase and LDLH, tumour-marker characteristics which have been reported to be significant prognostic factors in metastatic NPC, often associated with EBV infection (Jin et al., 2012). Table 1 summarizes the most significant associations.

These few examples clearly indicate that miRNA functional analysis tools can be of great help in studying the effects of circulating viral miRNAs, allowing the production of plausible hypotheses about their function and involvement in crucial cellular pathways, encouraging the development of more specific tools for computational investigation of cellular and extracellular viral miRNAs.

REFERENCES

Alexandrov, P. N., Dru, P., Hill, J. M., Bhattacharyya, S., Zhao, L. and Luhow, W. J. (2012). microRNA (miRNA) expression in Alzheimer’s disease (AD) cerebrospinal fluid (CSF) and extracellular fluid (ECF). J. Alzheimers Dis. 33, 365–373.

Arroyo, J. D., Chevillet, J. R., Kroh, E. M., Bal, I. K., Prindiville, C. C., Gibson, D. F., et al. (2011). Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc. Natl. Acad. Sci. U.S.A. 108, 5003–5008. doi: 10.1073/pnas.1009053108

Barth, D. P. (2009). MicroRNA target recognition and regulatory functions. Curr. Opin. 15, 215–225. doi: 10.1016/j.cell.2009.01.002

Barman, Y., Nachman, D., Viskinis, A., Tuskanian, P., Drayman, N., Stum-Ginossar, N., et al. (2011). An identical miRNA of the human JCV and BK polyoma viruses targets the stress-induced ligand and ubiquitin to escape immune elimination. Cell Host Microbe 9, 85–92. doi: 10.1016/j.chom.2011.01.008

Bos, R. A., and Vickers, K. C. (2015). Interconnect transport of microRNAs. Antiviral. Thromb. Vas. Biol. 53, 186–192. doi: 10.1111/avvb.12038

Carl, J. W., Jr., Trogvich, J., and Husaini, N., Stern-Ginossar, N., et al. (2011). Interconnect transport of microRNAs. Antiviral. Thromb. Vas. Biol. 53, 186–192. doi: 10.1111/avvb.12038

Chen, J. K. (2009). Human immunodeficiency virus type 1 Nef protein targets CD4 to the multivesicular body pathway. J. Virol. 83, 6578–6586. doi: 10.1128/JVI.00548-09
Everly, D. N. Jr., Kusano, S., and Laganà et al. Extracellular circulating viral microRNAs
Gallo, A., T andon, M., Alevizos, I., Altuvia, Y. (2011). RepT ar: a database of predicted cellular targets of host and viral miRNAs.
Hofree, M., Margalit, H., and Dawson, V. L. (2009). Understanding miRNA relevance? J. Virol. 83, 102, 4336–4344.
Jin, Y., Fan, X., Yi, C., Cao, Y., Xia, Q., Tan, Y. T. et al. (2012). To build a prognostic score model containing indoluble tumor markers for metastatic nephroblastoma carcinomas in an epidemic area. Eur. J. Cancer 48, 882–888. doi: 10.1016/j.ejca.2011.09.004
Kaul, D., Alkawar, A., and Gupta, S. D. (2009). HIV-1 genome-encoded hirL-miR1 impacts cellular responses to infection. Mol. Cell. Biochem. 323, 143–146. doi: 10.1007/s11010-009-9977-4
Kre, K. M., Laurin, K. M., and Kieff, E. (1995). Epstein-Barr virus latent membrane protein 1 is essential for B-lymphocyte growth transformation. J. Virol. 69, 729–731. doi: 10.1128/97498
Kun, P. L., Wang, Y., Zhou, J., Zhou, S., and Panaretakis, T. (2012). Tumor cell-derived exosomes: a message in the bottle. Cell. Mol. Life Sci. 69, 686–693. doi: 10.1007/s00705-011-1181-y
Lee, S. H., Kaleita, R. F., Kerry, J., Sommers, O. J., O’Connor, C. M., Khan, Z. F., et al. (2012). BdBK1 restriction factor is neutralized by proteasomal degradation and microRNA expression during human cytomegalovirus infection. Proc. Natl. Acad. Sci. U.S.A. 109, 9575–9580. doi: 10.1073/pnas.1207484109
Li, S. C., Shua, C. K., and Lin, W. C. (2008). Micro-RNA as tumor marker: microRNA-155 in exosomes. Proc. Natl. Acad. Sci. U.S.A. 105, 10772–10777.
Ling, J. D., Batto, P., Ribeiro, R., Battey, J. H., and Nusrat, A. (2004). Accumulation of lymphoendocrine antigen and natural killer cell lineage gene products in Epstein-Barr virus-infected cells. J. Virol. 78, 11608–11615. doi: 10.1128/JVI.78.21.11648-11655.2004
Laganà, A., Forte, S., Russo, F., Giugno, M., Erkizia, I., Puertas, M. C., Borin, F. F., Blanco, J., et al. (2010). Prediction of human targets for viral-encoded microRNAs by thermodynamics and empirical constraints. J. Virol. Genomes 6, 379–385.
Lee, S. H., Kaleita, R. F., Kerry, J., Sommers, O. J., O’Connor, C. M., Khan, Z. F., et al. (2012). BdBK1 restriction factor is neutralized by proteasomal degradation and microRNA expression during human cytomegalovirus infection. Proc. Natl. Acad. Sci. U.S.A. 109, 9575–9580. doi: 10.1073/pnas.1207484109
Liang, D., Fort, S., Battino, M., and Ghanezadeh, N. et al. (2011). The role of exosomes in intestinal inflammation. Cell. Mol. Life Sci. 68, 392–400. doi: 10.1007/s00705-011-1181-y
Li, S. C., Shua, C. K., and Lin, W. C. (2008). Micro-RNA as tumor marker: microRNA-155 in exosomes. Proc. Natl. Acad. Sci. U.S.A. 105, 10772–10777.
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Li, S. C., Shua, C. K., and Lin, W. C. (2008). Micro-RNA as tumor marker: microRNA-155 in exosomes. Proc. Natl. Acad. Sci. U.S.A. 105, 10772–10777.
Ling, J. D., Batto, P., Ribeiro, R., Battey, J. H., and Nusrat, A. (2004). Accumulation of lymphoendocrine antigen and natural killer cell lineage gene products in Epstein-Barr virus-infected cells. J. Virol. 78, 11608–11615. doi: 10.1128/JVI.78.21.11648-11655.2004
Laganà, A., Forte, S., Russo, F., Giugno, M., Erkizia, I., Puertas, M. C., Borin, F. F., Blanco, J., et al. (2010). Prediction of human targets for viral-encoded microRNAs by thermodynamics and empirical constraints. J. Virol. Genomes 6, 379–385.
and posttransplant lymphoproliferative disorder in a child with severe combined immunodeficiency: case report and review of the literature. Pediatr Dev Pathol 6, 449–457. doi: 10.1007/s10024-006-0300-7

Martinelli, A., Lerro, G., Ahlstrom, T., Stredny, R. W., J. Sullivan, M. L., Stolz, D. R., Papworth, G. D., et al. (2004). Endocytic, extracellular, cellular, and processing of exosomes by dendritic cells. Blood 104, 3257–3266. doi: 10.1182/blood-2004-03-1842

Morrison, J. A., Klingelhofer, A. J., and Raab-Traub, N. (2001). Epstein-Barr virus latent membrane protein is selectively secreted into the extracellular environment via exosomes. Exp Cell Res 266, 28–37. doi: 10.1006/EXCR.2001.4421

Morelli, A. E., Larregina, A. T., Shufesky, P., Pegtel, D. M., Cosmopoulos, K., Novellino, L., Rossi, R. L., Bonino, W. J., Sullivan, M. L., Stolz, D. B., et al. (2008). Epstein-Barr virus-associated immune dysregulation and Wnt/beta-catenin signaling through inhibition of WTX and promotes nasopharyngeal dysplasia but not tumorigenesis in LMP1(0/0) transgenic mice. J Virol 82, 253–266. doi: 10.1128/JVI.01386-07

Nerlov, L., Ross, R. L., Roman, F., Cordovil, A., Abribat, S., Paganu, M., et al. (2012). Circulating hepatic B surfaces antigen particles carry hepatocellular microRNAs. Proc Natl Acad Sci U S A 109, 13547–13552. doi: 10.1073/pnas.1213192

Palma, J., Yaldapar, S. C., Piggott, L., S. Weiner, G. M., et al. (2012). MicroRNAs are excluded from malignant cells in cultured tumors. Nucleic Acids Res 40, 9125–9138. doi: 10.1093/nar/gks714

Pegtel, D. M., Cosmopoulos, K., Thery-Lawson, D. A., van Eduard, M. A., Hopman, E. S., Lindingsr, J. L., et al. (2010). Functional asymmetric shedding of exosomes via proteins. Proc Natl Acad Sci U S A 107, 6327–6332. doi: 10.1073/pnas.1001360

Sjöstrand, M., Lee, J. J., and Lötvall, J. (2010). Exosome-mediated transfer of mRNAs and microRNAs is a new mode of macromolecular transport between cells. PLoS ONE 5, e13247. doi: 10.1371/journal.pone.0001324

Seo, G. J., Chen, C. J., and Sullivan, C. S., Grundhoff, A. T., et al. (2011). Altered profile of seminal microRNA in ejaculate from men with severe varicocele. PLoS ONE 6, e19024. doi: 10.1371/journal.pone.0021902

Trinchieri, G., Elenkov, I. G., and Seglen, P. O. (2000). Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 3, 183–190. doi: 10.1038/35047510

Uhlin, B., Sjöqvist, H., Boci, S., Nigita, G., Macca, R., et al. (2008). Identification of virus-encoded microRNAs of Epstein-Barr virus. J Virol 82, 295–304. doi: 10.1128/JVI.01531-07

Yang, C., Yao, B., Yang, C., Zhu, C., et al. (2012). An EBV-encoded microRNA that is overexpressed in immortalized lympho-histiocytic cell lines. J Exp Med 209, 1222–1231. doi: 10.1084/jem.20111747

Zernecke, A., Böddicker, K., Niola, H., Shagdarsuren, E., Gan, L., Demich, B., et al. (2009). Delivery of microRNA-126 by apoptotic bodies induces CX3CL1-dependent vascular protection. Arterioscler Thromb Vasc Biol 29, 212–218. doi: 10.1161/ATVBAHA.108.182063

Zhao, X., Xue, Z., Yu, C., Zhang, X., and Zhang, X. E. (2012). Functional orthologous viral and cellular microRNAs studied by a novel dual-fluorescent reporter system. PLoS ONE 7, e55247. doi: 10.1371/journal.pone.0055247

Zou, X., Zhang, Z., Fan, J., Cai, Z., and Zhang, X. E. (2012). Functional orthologous viral and cellular microRNAs studied by a novel dual-fluorescent reporter system. PLoS ONE 7, e55247.
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