Chapter 5

Prediction of Ebolavirus Genomes Encoded MicroRNA-Like Small RNAs Using Bioinformatics Approaches

Yue Teng, Zhe Xu, Jin Yuan, Xiaoping An, Jiangman Song and Dan Feng

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62944

Abstract

Recent findings revealed that certain viruses encoded microRNA-like small RNAs using the RNA interference machinery in the host cells. However, the function of these virus-encoded microRNA-like small RNAs remained unclear, and whether these microRNA-like small RNAs were involved in the replication of the virus and viral infection was still disputable. In this chapter, the negative-sense RNA genome of Ebola virus (EBOV) was scanned using bioinformatics tools to predict the EBOV-encoded microRNA-like small RNAs. Then, the potential influence of viral microRNA-like small RNAs on the viral immune evasion, host cellular signaling pathway, and epigenetic regulation of antiviral defense mechanism were also detected by the reconstructed regulatory network of target genes associated with viral encoded microRNA-like small RNAs. In this analysis, EBOV-encoded microRNA-like small RNAs were proposed to inhibit the host immune response factors, probably facilitating the evasion of EBOV from the host defense mechanisms. In conclusion, systematic investigation of microRNA-like small RNAs in EBOV genome may shed light on the underlying molecular mechanisms of the pathological process of Ebola virus disease (EVD).

Keywords: Ebolavirus, virus-encoded miRNAs, microRNAs, bioinformatics, NF-kB, TNF

1. Introduction

Zaire Ebola virus (ZEBOV) has the highest case-fatality rate with an average of approximately 83% over the past 27 years [1]. Its first outbreak took place on August 26, 1976, in Yambuku [2], and the virus was also responsible for the 2014 West Africa outbreak, which was the largest

EBOV outbreak in record [3–6]. Moreover, neither antiviral drugs nor effective treatment was available for EBOV or Ebola virus disease (EVD) at that time [7, 8]. MicroRNAs originate from a wide variety of primary transcripts (pri-miRNAs) that are generated by RNA polymerase II (pol II) in all eukaryotes [9] or by RNA polymerase III (pol III) in some viruses [10]. The cleavage of pri-miRNAs releases a RNA hairpin intermediate (~70 nt) containing a characteristic 2 nt 3′ overhang, named a premature miRNA (pre-miRNA), which is further processed to generate the 21~23 nt mature miRNA from its arm of ~70 nt imperfect stem-loop structure [11, 12].

Since microRNAs have been discovered and their role in gene expression regulation was established, it has been hypothesized that viruses could encode microRNA-like small RNAs as well, and these virus-encoded microRNA-like small RNAs were proposed to play important regulatory roles in viral immune evasion and systemic pathogenesis [13–15]. The size of viral encoded microRNA-like RNAs has a significant advantage given the tight constraints on viral genome size, which is also small enough to escape from the triggered host immune pathway. It was found that viral encoded microRNA-like small RNAs could downregulate the expression of host immune defense gene, resulting in increased viral replication or evasion from host immune surveillance [16, 17]. Until now, more than 60 viral microRNA-like small RNAs have been identified [18–24], most of which came from Herpes virues [25]. Only a small part of such RNAs was detected within Retrovirus, Adenovirus, and polyomavirus families [26–28].

Bioinformatics-driven prediction was an effective method to identify viral encoded microRNA-like small RNAs [21, 22]. In this study, the microRNA prediction program, VMir, was applied to scan the viral genomes for the presence of stem-loop structures in the pri- and pre-miRNAs and identify potential candidate stretches capable to form stable secondary stem-loop structures. Afterward, putative mature microRNA-like small RNAs were validated using MatureBayes [29]. The systemic prediction of the potential EBOV-encoded microRNA-like small RNAs along with their target genes on the genome-wide scale helps to further assess the function of microRNAs during viral infection and virus-host interactions in the EVD pathogenesis.

2. Methods

2.1. EBOV whole genome sequences and alignment

The full-length genome sequences of EBOV were retrieved from the genome browser at Ebola virus resource (http://www.ncbi.nlm.nih.gov/genome/viruses/variation/ebola/) and UCSC Ebola portal (https://genome.ucsc.edu/ebolaPortal/). MAFFT Multiple Sequence Alignment Software Version 7 were applied for the alignment of the EBOV genomes [30].

2.2. Bioinformatics prediction of the EBOV genome-encoded microRNA-like small RNAs

Briefly, the viral genome was scanned for stem-loop structures of miRNA precursor (pre-miRNA) using VMir [31] with default parameter settings (http://www.hpi-hamburg.de/research/departments-and-research-groups/antiviral-defense-mechanism/software-down-

http://dx.doi.org/10.5772/62944

load.html). The putative pre-miRNAs with VMir score ≥150 and a window count ≥ 35 were retained. Then, MiPred software [32] was applied to check all of the putative miRNA precursors, and precursors with confidence equal to or greater than 70% were further analyzed. Subsequently, mature miRNA sequences were predicted from the putative pre-miRNA stem-loops. Finally, the MatureBayes tool [29] was used to extend the prediction coverage of the mature miRNAs under default parameter settings.

2.3. Prediction of the target genes and signaling pathway analysis

Target genes of predicted EBOV-encoded microRNA-like small RNAs in the human genome were predicted using TargetScan [33]. Putative targets within the viral genome were predicted using TargetScan Perl script. The signaling pathways collected from the Kyoto Encyclopedia of Genes and Genomes (KEGG) [34–36] PATHWAY databases were applied in the pathway analysis.

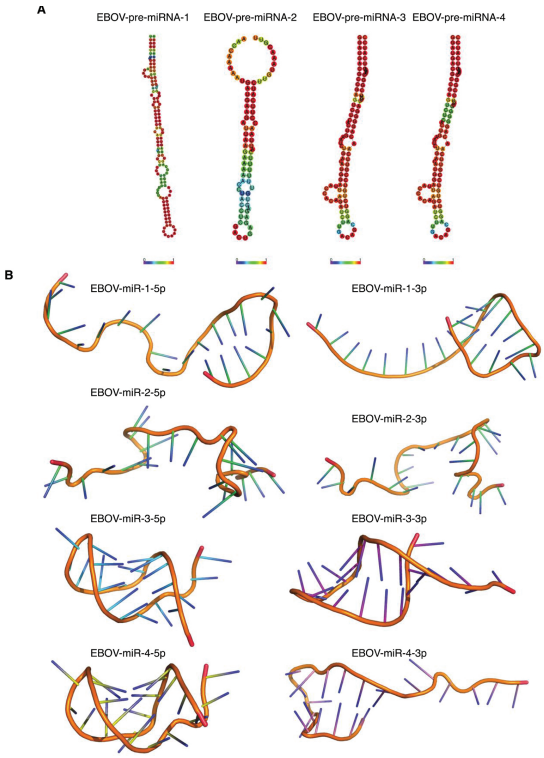

Figure 1. The predicted EBOV-encoded pre-miRNAs and microRNA-like small RNAs. The MiPred algorithm was used to identify genuine pre-miRNAs, and the MatureBayes tool was used to predict the mature miRNA sequences. (A) The secondary structures of the four EBOV pre-miRNAs. (B) The tertiary structures of the EBOV-encoded microRNA-like small RNAs.

2.4. Constructing gene regulation network

The genetic regulation network was constructed based on systematic integration of various datasets. Transcription factors related with the target genes of EBOV-encoded microRNA-like small RNAs were selected from Transcriptional Regulatory Element Database (TRED) [37–39]. The integrated regulatory network of target genes with transcription factors was constructed using Cytoscape software (http://cytoscape.org/).

3. Key findings regarding the bioinformatics prediction of EBOV genome-encoded microRNA-like small RNAs

3.1. Predicted precursor and mature EBOV genome-encoded microRNA-like small RNAs

The released full-length genome sequences of the retrieved EBOV strains were aligned and then scanned for miRNA precursor (pre-miRNA) using VMir software. Afterward, the putative pre-miRNAs with VMir score ≥150 and a window count ≥35 were selected for further assessment. Within the EBOV genome, four putative microRNA precursors, EBOV-pre-miRNA-1, EBOV-pre-miRNA-2, EBOV-pre-miRNA-3, and EBOV-pre-miRNA-4 were predicted (**Figure 1A**). The mature miRNA sequences were predicted from the putative pre-miRNA stem loops. Seven different mature EBOV miRNA candidates, including EBOV-miR-1-5p, EBOV-miR-1-3p, EBOV-miR-2-5p, EBOV-miR-2-3p, EBOV-miR-3-5p, EBOV-miR-3-3p, EBOV-miR-4-5p, and EBOV-miR-4-3p were resolved using MatureBayes tool (**Figure 1B**).

3.2. Bioinformatics analysis of the genetic regulation network in the target genes of EBOV genome-encoded microRNA-like small RNAs

Target genes of the predicted mature microRNA-like small RNAs were searched within TargetScan, and the potential target genes in host were identified (**Table S1**, the list of potential target genes of EBOV-encoded microRNA-like small RNAs). KEGG pathway enrichment analysis was performed using the DAVID bioinformatics tool for these target genes. The results showed that the target genes were closely related on function and were involved in multiple canonical pathways, such as NF-kB activation by viruses, role of protein kinase (PKR) in interferon induction and antiviral response, induction of apoptosis by HIV1, B cell-activating factor signaling, and role of PI3K/AKT signaling in the pathogenesis of influenza, which were important in human immune response to virus infection (**Table 1**).

Canonical pathways	p-Value	Ratio	Molecules
AMPK signaling	*1.49E+00*	*2.26E-02*	*PDRK1, FASN, ADRA2B, RRKAB2*
Angiopoietin signaling	*4.47E-01*	*1.54E-02*	*NFKBIE*
April mediated signaling	*6.43E-01*	*2.63E-02*	*NFKBIE*
ATM signaling	*4.81E-01*	*1.69E-02*	*MRE11A*

http://dx.doi.org/10.5772/62944

Canonical pathways	p-Value	Ratio	Molecules
B cell activating factor signaling	*6.24E-01*	*2.5E-02*	*NFKBIE*
B cell receptor signaling	*4.92E-01*	*1.17E-02*	*PDPK1, NFKBIE*
CD27 signaling in lymphocytes	*5.34E-01*	*1.96E-02*	*NFKBIE*
CD28 signaling in t helper cells	*7.5E-01*	*1.77E-02*	*PDPK1, NFKBIE*
CD40 signaling	*4.53E-01*	*1.56E-02*	*NFKBIE*
bf2 signaling	*4.89E-01*	*1.16E-02*	*PDPK1, EIF2AK4*
ErbB signaling	*3.58E-01*	*1.18E-02*	*PDPK1*
ErbB2-ErbB3 signaling	*5E-01*	*1.79E-02*	*PDPK1*
ErbB4 signaling	*4.87E-01*	*1.72E-02*	*PDPK1*
Erythropoietin signaling	*1.12E+00*	*2.99E-02*	*PDPK1, NFKBIE*
HGF signaling	*2.95E-01*	*9.62E-03*	*ELF3*
HIF1a signaling	*3.07E-01*	*1E-02*	*MMP25*
IGF-1 signaling	*1.55E+00*	*3.09E-02*	*GRB10, PDPK1, SOCS4*
IL-1 signaling	*8.99E-01*	*2.2E-02*	*GNAT1, NFKBIE*
IL-10 signaling	*4.32E-01*	*1.47E-02*	*NFKBIE*
IL-17A signaling in airway cells	*4.53E-01*	*1.56E-02*	*NFKBIE*
IL-17A signaling in fibroblasts	*6.75E-01*	*2.86E-02*	*NFKBIE*
il-6 signaling	*2.63E-01*	*8.62E-03*	*NFKBIE*
Induction of apoptosis by HIV1	*1.22E+00*	*3.39E-02*	*NFKBIE, RIPK1*
Insulin receptor signaling	*6.69E-01*	*1.56E-02*	*GRB10, PDPK1*
JAK/Stat signaling	*4.12E-01*	*1.39E-02*	*SOCS4*
Lymphotoxin β receptor signaling	*5.13E-01*	*1.85E-02*	*PDPK1*
MIF regulation of innate immunity	*6.14E-01*	*2.44E-02*	*NFKBIE*
mTOR signaling	*4.57E-01*	*1.1E-02*	*PDPK1, PRKAB2*
NF-KB activation by viruses	*1.06E+00*	*2.74E-02*	*NFKBIE, RIPK1*
NF-KB signaling	*4.99E-01*	*1.18E-02*	*NFKBIE, RIPK1*
NGF signaling	*2.89E-01*	*9.43E-03*	*PDPK1*
P53 signaling	*3.13E-01*	*1.02E-02*	*CCND2*
PI3K signaling in B lymphocytes	*6.94E-01*	*1.63E-02*	*PDPK1, NFKBIE*
PI3K/AKT signaling	*7.05E-01*	*1.65E-02*	*PDPK1, NFKBIE*
PKCθ signaling in T lymphocytes	*2.71E-01*	*8.85E-03*	*NFKBIE*
PPARa/RXRa activation	*9.95E-01*	*1.82E-02*	*FASN, NFKBIE, PRKAB2*
Regulation of IL-2 expression in activated and anergic T lymphocytes	*3.86E-01*	*1.28E-02*	*NFKBIE*

Canonical pathways	p-Value	Ratio	Molecules
Role of IL-17A in arthritis	*5.13E-01*	*1.85E-02*	*NFKBIE*
Role of NFAT in regulation of the immune response	*5.1E-01*	*1.2E-02*	*GNAT1, NFKBIE*
Role of PI3K/AKT signaling in the pathogenesis of influenza	*4.75E-01*	*1.67E-02*	*NFKBIE*
Role of PKR in interferon induction and antiviral response	*6.24E-01*	*2.5E-02*	*NFKBIE*
STAT3 pathway	*4.08E-01*	*1.37E-02*	*SOCS4*
TNFR1 signaling	*1.4E+00*	*4.26E-02*	*NFKBIE, RIPK1*
TNFR2 signaling	*7.62E-01*	*3.57E-02*	*NFKBIE*
AMPK signaling	*1.49E+00*	*2.26E-02*	*PDPK1, FASN, ADRA2B, PRKAB2*
Angiopoietin signaling	*4.47E-01*	*1.54E-02*	*NFKBIE*
April mediated signaling	*6.43E-01*	*2.63E-02*	*NFKBIE*
ATM signaling	*4.81E-01*	*1.69E-02*	*MRE11A*
B cell activating factor signaling	*6.24E-01*	*2.5E-02*	*NFKBIE*
B cell receptor signaling	*4.92E-01*	*1.17E-02*	*PDPK1, NFKBIE*
CD27 signaling in lymphocytes	*5.34E-01*	*1.96E-02*	*NFKBIE*
CD28 signaling in T helper cells	*7.5E-01*	*1.77E-02*	*PDPK1, NFKBIE*
CD40 signaling	*4.53E-01*	*1.56E-02*	*NFKBIE*
EIF2 signaling	*4.89E-01*	*1.16E-02*	*PDPK1, EIF2AK4*
ErbB signaling	*3.58E-01*	*1.18E-02*	*PDPK1*
ErbB2-ErbB3 signaling	*5E-01*	*1.79E-02*	*PDPK1*
ErbB4 signaling	*4.87E-01*	*1.72E-02*	*PDPK1*
Erythropoietin signaling	*1.12E+00*	*2.99E-02*	*PDPK1, NFKBIE*
HGF signaling	*2.95E-01*	*9.62E-03*	*ELF3*
HIF1a signaling	*3.07E-01*	*1E-02*	*MMP25*
IGF-1 signaling	*1.55E+00*	*3.09E-02*	*GRB10, PDPK1, SOCS4*
IL-1 signaling	*8.99E-01*	*2.2E-02*	*GNAT1, NFKBIE*
IL-10 signaling	*4.32E-01*	*1.47E-02*	*NFKBIE*
IL-17A signaling in airway cells	*4.53E-01*	*1.56E-02*	*NFKBIE*
IL-17A signaling in fibroblasts	*6.75E-01*	*2.86E-02*	*NFKBIE*
IL-6 signaling	*2.63E-01*	*8.62E-03*	*NFKBIE*
Induction of apoptosis by HIV1	*1.22E+00*	*3.39E-02*	*NFKBIE, RIPK1*

Table 1. Key canonical pathway analysis of the potential mature EBOV miRNA target genes.

http://dx.doi.org/10.5772/62944

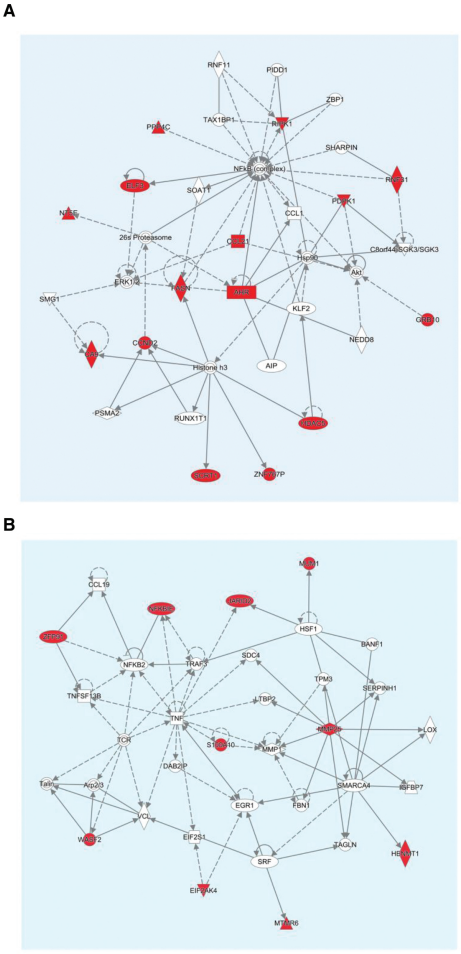

Figure 2. Bioinformatics analysis of the genetic regulatory network of target genes of EBOV-encoded microRNA-like small RNAs (A and B). The key regulation network of the potential target genes of EBOV-encoded microRNA-like small RNAs.

Based on the gene regulation network (GRN) analysis (Figure S1), it was found that target genes, FASN, RUNX1T1, and ELF3, were important immune and inflammation response factors and actively interacted with transcription regulator, such as KLF2 and NF-kB in host cells (**Figure 2A**) [40, 41]. They were also the key co-regulator of TNF complex in human immune system (**Figure 2B**) [42], implying that the EBOV might inhibit the infection response of immune system by affecting the related signaling pathway using noncoding RNA. Furthermore, it was speculated that the mature EBOV-encoded microRNA-like small RNAs might induce large-scale epigenetic modification in host genome to downregulate the expression of epigenetic factor, such as histone h3, HDAC5, JARID2, and SMARCA4, resulting

in the inactivation of immune signaling and immune system with the antiviral response (**Figure 2A** and **2B**) [40–45].

3.3. Potential EBOV genome-encoded microRNA-like small RNAs associated with the Immune response-related pathways

Additionally, NF-kB and RIPK were also involved in the RIG-I-like receptor pathway (**Figure 3**) [46, 47]. As shown in **Figure 3**, the RIG-I-like receptor pathway played a key role in antiviral response that is a sensor for viruses such as influenza A, Rhabdovirus, Flavivirus, Paramyxovirus, Epstein-Barr virus, and Filovirus [48]. The RIG-I-like receptor pathway is stimulated during RNA virus infection by the interaction between cytosolic RIG-I and viral RNA structures that contain short hairpin dsRNA and 5′ triphosphate (5′ppp) terminal structure. The EBOV might utilize the microRNA-like small RNAs to inhibit the RIG-I-like receptor pathway to evade the host defense mechanisms, or conversely to trigger apoptosis responses as a

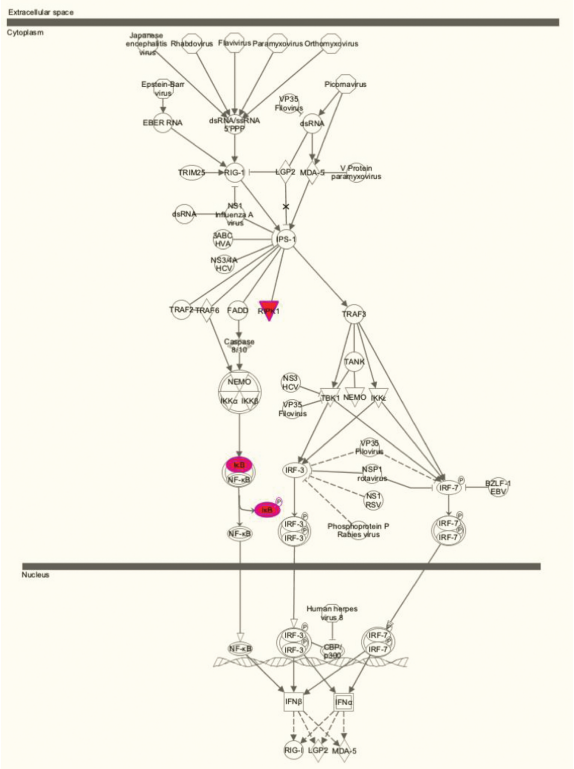

Figure 3. The RIG1I like receptor pathway associated with the potential target genes of EBOV-encoded microRNA1-like small RNAs. The target genes of EBOV-encoded microRNA1-like small RNAs, NF1kB, and RIPK, were involved in the RIG1I-like receptor pathway to trigger IFN signaling pathway with the antiviral response.

http://dx.doi.org/10.5772/62944

mechanism to increase viral infection [49, 50]. For viruses, effective RIG-I-mediated antiviral responses are dependent on functionally active LGP2. The dysfunction of LGP2 resulted in promoting viral replication, preventing virus-induced apoptosis, and suppressing the immune response for the invading pathogen [51]. Certain retroviruses, such as HIV-1, encode a protease that directs RIG-1 to the lysosome for degradation, and thereby evade RIG-1 mediated signaling. RIG-I and MDA-5 are involved in activating interferon (IFN) signaling pathway with the antiviral response.

4. Conclusions

MicroRNAs are encoded by cellular or viral genomes and play an essential role in numerous cellular processes, including viral infection, viral immune evasion, and antiviral cell-mediated immune response. Most viral genome-encoded microRNA-like small RNAs have been identified by traditional cloning strategy from virus-infected cells, yet others have been identified following computational prediction. Using the VMir analyzer program, the polyomavirus simian vacuolating virus 40 (SV40) [22] and Merkel Cell virus (MCV) [13] have been found to encode microRNA-like small RNAs, suggesting that VMir analyzer program is an effective tool for searching new viral miRNA-like small RNAs [52]. Therefore, we analyzed the genome of EBOV with the VMir software and obtained four pre-miRNAs located in the coding region of viral genome, indicating that the RNA secondary structures of EBOV genome might be processed into microRNA-like small RNAs [53, 54].

Infected cells have several signaling mechanisms to sense and respond to virus infection [55], for example, cross talk between different cellular pathways to modulate the expression and antiviral function of interferon (IFNs) with RIG-I-like receptor pathway and specific gene products. RIG-I-like receptor pathway and IFNs cytokines are important regulators of innate and adaptive immune responses [56]. Besides their antiviral role, they are potent regulators of cell growth and have immunomodulatory activity. INFs were activated after virus infection, probably through viral dsRNA and other viral gene products. The most intensely studied molecule in the RIG-I-like receptor pathway is the dsRNA-activated serine/threonine protein kinase (PKR). PKR was activated in the presence of cytoplasmic dsRNA, leading to the rapid phosphorylation of eukaryotic initiation factor eIF2 and subsequent inhibition of both host and viral mRNA [57, 58].

Although the bioinformatics prediction could be inaccurate, the bioinformatics prediction was potentially more selective and effective than experimental method. The target genes of viral genome-encoded microRNA-like small RNAs would help to develop an effective treatment for the EBOV infection.

5. Limitations

Due to the high mutation rate of reverse transcription in replication, EBOV presents numerous mutations over viral genome during host adaption, suggesting that the viral genome is not

exactly the same among various EBOV strains. Thus, it is difficult to find microRNAs that are completely conserved among different viral strains due to genome mutations.

However, it is possible that some microRNA-like small RNAs are relatively conserved among diverse viral adapted hosts. Moreover, the expression pattern of viral microRNA-like small RNAs was highly unpredictable. Therefore, it might be difficult to validate the EBOV genome-encoded microRNA-like small RNAs using experimental method.

Acknowledgements

This work was supported by a grant from the State Key Laboratory of Pathogen and BioSecurity Program (No. SKLPBS1408 and No. SKLPBS1451).

Authors' contributions

Zhe Xu, Yuan Jin, and Xiaoping An characterized the materials, under the supervision of Yue Teng, Zhe Xu, and Dan Feng wrote the manuscript with further contributions from Jiangman Song and Yuan Jin analyzed the data. All authors reviewed the manuscript.

Conflict of interest

Competing financial interests and the authors declare no competing financial interests.

Author details

Yue Teng[1*], Zhe Xu[2], Jin Yuan[1], Xiaoping An[1], Jiangman Song[3] and Dan Feng[4]

*Address all correspondence to: yueteng@me.com

1 The State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China

2 Core Laboratory for Clinical Medical Research, Beijing Tiantan Hospital, Capital Medical University, Beijing, China

3 Department of Neurology, People's Hospital, Peking University, Beijing, China

4 Division of Standard Operational Management, Institute of Hospital Management, Chinese PLA General Hospital, Beijing, China

References

[1] Cenciarelli, O., Pietropaoli, S., Malizia, A., et al. Ebola virus disease 2013-2014 outbreak in west Africa: an analysis of the epidemic spread and response. *Int J Microbiol*. 2015, 769121 (2015).

[2] Ksiazek, T.G. Filoviruses: Marburg and Ebola. *Viral Infections of Humans*. 14, 337–350 (2014).

[3] World Health Organization. "Global Alert and Response (GAR): Situation Reports: Ebola Response Roadmap," 2015. Available at: http://www.who.int/csr/disease/ebola/situation-reports/en/.

[4] World Health Organization. "Ebola Situation Reports," 2014. Available at: http://apps.who.int/ebola/ebola-situation-reports.

[5] Frieden, T.R., Damon, I., Bell, B.P., Kenyon, T. and Nichol, S. Ebola 2014 – new challenges, new global response and responsibility. *N Eng J Med*. 371, 1117–1180 (2014).

[6] Walker, N.F., Whitty, C.J. Tackling emerging infections: clinical and public health lessons from the West African Ebola virus disease outbreak, 2014–2015. *Clin Med*. 15, 457–460 (2015).

[7] Alexander, K.A., Sanderson, C.E., Marathe, M., et al. What factors might have led to the emergence of Ebola in West Africa? *PLoS Negl Trop Dis*. 9(6), e0003652 (2015).

[8] Turner, C. Ebola virus disease: An emerging threat. *Nursing*. 44, 68–69 (2014).

[9] Kim, V. N., Han, J. and Siomi, M.C. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol*. 10, 128–139 (2009).

[10] Grundhoff, A. and Sullivan, C.S. Virus-encoded microRNAs. *Virology*. 411, 325–343 (2011).

[11] Brodersen P. and Voinnet, O. Revisiting the principles of microRNA target recognition and mode of action. *Nat Rev Mol Cell Biol*. 10, 141–148 (2009).

[12] Carthew, R.W. and Sontheimer, E.J. Origins and mechanisms of miRNAs and siRNAs. *Cell*. 136, 642–655 (2009).

[13] Seo, G.J., Chen, C.J. and Sullivan, C.S. Merkel cell polyomavirus encodes a microRNA with the ability to autoregulate viral gene expression. *Virology*. 383, 183–187 (2009).

[14] Pfeffer, S., et al. Identification of virus-encoded microRNAs. *Sicence*. 304, 734–736 (2004).

[15] Cullen, B.R. Viruses and microRNAs. *Nat Genet*. 38, S25–S30 (2006).

[16] Kincaid, R.P. and Sullivan, R.P. Virus-encoded microRNAls: an overview and a look to the future. *Plos Pathog*. 8, e10033018 (2012). DOI: 10.1371/journal.ppat.1003018.

[17] Lecellier, C.H., et al. A cellular microRNA mediates antiviral defense in human cells. *Science*. 308, 557–560 (2005).

[18] Walz, N., Christalla, T., Tessmer, U. and Grundhoff, A. A global analysis of evolutionary conservation among known and predicted gammaherpesvirus microRNAs. *J. Virol*. 84, 716–728 (2010).

[19] Sullivan, C.S., et al. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. *Nature*. 435, 682–686 (2005).

[20] Samols, M.A., Hu, J., Skalsky, R.L. and Renne, R. Cloning and identification of a microRNA cluster within the latency-associated region of Kaposi's sarcoma-associated herpesvirus. *J. Virol*. 79, 9301–9305 (2005).

[21] Cui, C. Prediction and identification of herpes simplex virus 1-encoded microRNAs. *J. Virol*. 80, 5499–5508 (2006).

[22] Sullivan, C.S., Grundhoff, A.T., Tevethia, S., Pipas, J.M. and Ganem, D. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cell. *Nature*. 435, 682–686 (2005).

[23] Cai, X., et al. Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathog*. 2, e23 (2006).

[24] Liang, H. Identification of Ebola virus microRNAs and their putative pathological function. *Sci China Life Sci*. 57, 973–981 (2014).

[25] Pfeffer, S., et al. Identification of microRNAs of the Herpesvirus family. *Nat. Methods*. 2, 269–276 (2005).

[26] Omoto, S., et al. HIV-1 nef suppression by virally encoded microRNA. *Retrovirology*. 1, 44 (2004).

[27] Bennasser, Y., et al. Evidence that HIV-1 encodes an siRNA and a suppressor of RNA silencing. *Immunity*. 22, 607–619 (2005).

[28] Rosewick, N. Deep sequencing reveals abundant noncanonical retroviral microRNAs in B-cell leukemia/lymphoma. *Proc. Natl. Acad. Sci. USA*. 110, 2306–2311 (2013).

[29] Gkirtzou, K., Tsamardinos, I., Tsakalides P. and Poirazi, P. MatureBayes: a probabilistic algorithm for identifying the mature miRNA within novel precursors. *Plos One*. 5, e11843 (2010). DOI: 10.1371/journal.pone.0011843.

[30] Katoh, K. and Standley, D.M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 30, 772–780 (2013).

[31] Li, S.C., Shiau, C.K. and Lin, W.C. Vir-Mir db: prediction of viral microRNA candidate hairpins. *Nucleic Acids Res*. 36, 184–189 (2008).

[32] Jiang, P. MiPred: classification of real and pseudo microRNA precursors using random forest prediction model with combined features. *Nucleic Acids Res*. 35, 339–344 (2007).

[33] Sethupathy, P., Corda, B. and Hatziqeorqiou, A.G. TarBase: a comprehensive database of experimentally supported animal microRNA targets. *RNA*. 12, 192–197 (2006).

[34] Kanehisa, M. and Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 28, 27–30 (2000).

[35] Huang, D.W., Sherman, B.T. and Lempicki, R.A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc*. 4, 44–57 (2009).

[36] Mi, H., Muruganujan, A. and Thomas, P.D. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. *Nucleic Acids Res*. 41, 377–386 (2013).

[37] Mi, H., Muruganujan, A., Casaqrande, J.T. and Thomas, P.D. Large-scale gene function analysis with the PANTHER classification system. *Nat Protoc*. 8, 1551–1566 (2013).

[38] Franceschini, A., et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. *Nucleic Acids Res*. 41, 808–815 (2013).

[39] Huang, D.W., Sherman, B.T. and Lempicki, R.A. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*. 37, 1–13 (2009).

[40] Bayarsaihan, D. Epigenetic mechanisms in inflammation. *J. Dent. Res*. 90, 9–17 (2011).

[41] Hayden, M.S., West, A.P. and Ghosh, S. NF-kappaB and the immune response. *Oncogene*. 25, 6758–6780 (2006).

[42] Bouwmeester, T. A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. *Nat. Cell Biol*. 6, 97–105 (2004).

[43] Xu, W. Ebola virus VP24 targets a unique NLS binding site on karyopherin alpha 5 to selectively compete with nuclear import of phosphorylated STAT1. *Cell Host Microbe*. 16, 187–200 (2014).

[44] Kagoya, Y. Positive feedback between NF-kB and TNF-alpha promotes leukemia-initiating cell capacity. *J. Clin. Invest*. 124, 528–542 (2014).

[45] Lee, R.E., Walker, S.R., Savery, K., Frank, D.A. and Gaudet, S. Fold change of nuclear NF-kB determines TNF-induced transcription in single cells. *Mol. Cell*. 53, 867–879 (2014).

[46] Ramos, H.J. and Gale, M., Jr. RIG-I like receptors and their signaling crosstalk in the regulation of antiviral immunity. *Curr Opin Virol*. 1, 167–176 (2011).

[47] Loo, Y.M. and Gale, M., Jr. Immune signaling by RIG-I-like receptors. *Immunity*. 34, 680–692 (2011).

[48] Solis, M., et al. RIG-I-mediated antiviral signaling is inhibited in HIV-1 infection by a protease-mediated sequestration of RIG-I. *J Virol*. 85, 1224–1236 (2011).

[49] Pichlmair, A., et al. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. *Science*. 314, 997–1001 (2006).

[50] Gupta, A., et al. Anti-apoptotic function of a microRNA encoded by the HSV-1 latency-associated transcript. *Nature*. 442, 82–85 (2006).

[51] Satoh, T., et al. LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. *Proc Natl Acad Sci U S A*. 107, 1512–1517 (2010).

[52] Shi, J. Identification and validation of a novel microRNA-like molecule derived from a cytoplasmic RNA virus antigenome by bioinformatics and experimental approaches. *Virol. J*. 11, 121:1–121:14 (2014).

[53] Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res*. 31, 3406–3415 (2003).

[54] Parisien, M. and Major, F. The MC-fold and MC-sym pipeline infers RNA structure from sequence data. *Nature*. 452, 51–55 (2008).

[55] Randall, R.E. and Goodbourn, S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J. Gen. Virol*. 89, 1–47 (2008).

[56] Kaul, D., Ahlawat, A. and Gupta, S.D. HIV-1 genome-encoded HIV1-MIR-H1 impairs cellular responses to infection. *Mol. Cell Biochem*. 323, 143–148 (2009).

[57] Hussain, M. West Nile virus encodes a microRNA-like small RNA in the 3' untranslated region which up-regulates GATA4 mRNA and facilitates virus replication in mosquito cells. *Nucleic Acids Res*. 40, 2210–2223 (2012).

[58] Hussain, M., Taft, R.J. and Asgari, S. An insect virus-encoded microRNA regulates viral replication. *J. Virol*. 82, 9164–9170 (2008).