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-

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