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MicroRNA profile analysis of a feline kidney cell line before and after infection with mink enteritis virus

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ABSTRACT

MicroRNAs (miRNAs) are small regulatory RNAs that play a significant role in eukaryotes by targeting mRNAs for cleavage or translational repression. Recent studies have also shown them to be associated with cellular changes following viral infection. Mink enteritis virus (MEV) is one of the most important viral pathogens in the mink industry. To study the involvement of miRNAs in the MEV infection process, we used Illumina's ultrahigh throughput approach to sequencing miRNA libraries from the feline kidney (F81) cell line before and after infection with MEV. Using this bioinformatics approach we identified 196 known mammalian miRNA orthologs belonging to 152 miRNA families in F81 cells. Additionally, 97 miRNA*s of these miRNAs were detected. As well as known miRNAs, 384 and 398 novel miRNA precursor candidates were identified in uninfected and MEV-infected F81 cells respectively that have not been reported in other mammals. In MEV-infected cells 3 miRNAs were significantly down-regulated and 4 up-regulated including 3 significantly. The majority (12 of 16) of randomly selected miRNA expression profiles by qRT-PCR were consistent with those identified by deep sequencing. A total of 88 miRNAs were predicted to target interferon-associated genes; 6 appear to target the 3′UTR of MEV-specific receptor transferring receptor mRNAs; and 8 to target the MEV mRNA coding region. No miRNAs coded by MEV itself were detected.

1. Introduction

MiRNAs are endogenous small RNAs of about 22 nucleotides (nt) that regulate gene expression by sequence-specific targeting of the 3′ UTR of mRNAs in the RNA-induced silencing complex. They can affect many cellular functions, such as proliferation, hematopoiesis and development of the nervous system (Ambros, 2004; Bartel, 2007; Krol et al., 2010). Recent studies have also focused on the role of miRNAs as modulators of host cell-virus interaction networks (Scaria et al., 2006). Evidence suggests that not only host cells but also viruses can encode miRNAs (Lei et al., 2010; Lu et al., 2010; Song et al., 2010). Since propagation of a virus is highly dependent on its host cell, the complex cellular regulatory network can have a major effect on viral replication. Cellular regulators such as miRNAs play a role in the self-protection process (Lagos et al., 2010). To combat these, some viruses respond via a miRNA–mRNA cross-talk function (Grey et al., 2010). The study of miRNA-mediated host-virus interactions therefore contribute to an understanding of the mechanism of virus infection and host counteraction.

Mink enteritis virus (MEV) is one of the most important viral pathogens in the mink industry. It is a single stranded DNA virus of the genus *Parvovirus* with a genome of about 5 kb (Zhang, 1997), both ends of which contain hairpin structures. The genome is comprised mainly of 2 open reading frames, the left coding for proteins NS1 and NS2 and the right for VP1 and VP2. VP2 is the major structural protein of the virion; NS1 is the main non-structural one. MEV infection results in a high rate of morbidity and mortality, exhibits a rapid clinical course and can spread rapidly (Rivera et al., 1987; Schofield, 1949; Zuo et al., 2010). Vaccines have been developed to prevent spread of the disease within animal facilities (Barker et al., 1983; Horiuchi et al., 1997; Langeveld et al., 1995; Parrish et al., 1982; Zhang, 1997).

Little research has been done using domestic cats (*Felis catus*) as an experimental animal or a source of cell cultures. The entire feline genome has, however, been sequenced (Pontius and O'Brien, 2007; Pontius et al., 2007). With this as a basis, therefore, our first aim was to construct a repertoire of miRNAs expressed in the F81 cell line, and to use this to study the responses of the latter to MEV infection. We utilized Illumina's ultrahigh throughput approach to sequencing and analysis of two miRNA libraries, from uninfected and MEV-infected cells.

Abbreviations: miRNA, microRNA; F81 cell line, feline kidney cell line; MEV, mink enteritis virus; nt, nucleotides; MEM, minimum essential medium; C3, uninfected cells; V3, virus-infected cells; PAGE, polyacrylamide gel electrophoresis; U, uridine; G, guanine; qRT-PCR, quantitative RT-PCR.

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2. Materials and methods

2.1. Virus and cells

MEV strain L (MEV-L) is a strain of MEV originally isolated from an infected mink in a mink farm, Liaoning province, China. The entire genome has been sequenced in our laboratory and found to be highly homologous with MEV strain Abashiri (GenBank accession, D00765.1).

F81 cells are feline kidney cells, obtained from the American Type Culture Collection (ATCC). For construction of small RNA libraries, F81 cells were cultured in 6-well plastic dishes (Costar) in minimum essential medium (MEM) (GIBCO, CA) containing 10% FBS (Hyclone, Logan, UT) and 1% penicillin–streptomycin (GIBCO, CA) at 37 °C in a 5% CO_2 atmosphere. Three wells of a dish were infected with MEV-L at an m.o.i. of 1 pfu/cell: the other 3 were left uninfected. At 24 h post-infection, virus-infected (V3) and uninfected (C3) cells were pooled separately for the following total RNA extraction.

2.2. Small RNA ultrahigh throughput sequencing and analysis of sequencing data (Glazov et al., 2008)

2.2.1. RNA preparation

Total RNAs of C3 and V3 samples were isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions, and quantitated by Nanodrop 2000 (Thermo). Two RNA samples were subjected to polyacrylamide gel electrophoresis (PAGE) for isolation of molecules of 18–30 nt length.

2.2.2. Small RNA library construction and sequencing

Small (18–30 nt) RNAs of each library, prepared from 10 μg of each RNA sample, were submitted to Solexa (Illumina Inc.) for the following sequencing. Proprietary adapters were ligated to both ends of the small RNA samples which were then transcribed to cDNA and amplified by 18 PCR cycles to produce sequencing libraries that were subjected to Solexa's proprietary sequencing-by-synthesis procedure.

2.2.3. Mapping the Solexa reads onto the feline genome

Adapter sequences were removed from both ends of the reads. All identical sequences were counted and duplication eliminated. The resulting set of unique sequences with associated read counts, referred to as sequence tags, were mapped onto the feline genome (Pontius and O'Brien, 2007; Pontius et al., 2007) using the program Short Oligonucleotide Analysis Package (SOAP) (Li et al., 2008).

2.2.4. Known miRNA identification

Perfectly matched reads were mapped onto miRNAs of six reference species (*Homo sapiens*, *Canis familiaris*, *Mus musculus*, *Rattus norvegicus*, *Bos taurus* and *Sus scrofa*) of the Sanger miRBase (Release 18) using the program Patscan (Bland et al., 2007).

Table 1
Primer sequences for miRNA qRT-PCR.

Primer names	Sequences (5′–3′)
U6 F	CTCGCTTCGGCAGCACA
U6 R	AACGCTTCACGAATTTGCGT
universal	GCGAGCACAGAATTAATACGACTCAC
adapter	GCGAGCACAGAATTAATACGACTCACTATAGGTTTTTTTTTTTTVN
miR-181a	AACATTCAACGCTGTCGGTGAGTA
miR-181b	AACATTCATTGCTGTCGGTGGG
miR-181c	AACATTCAACCTGTCGGTGAGTAAA
miR-181d	AACATTCATTGTTGTCGGTGGGTA
miR-23a	ATCACATTGCCAGGGATTTCCA
miR-99a	AACCCGTAGATCCGATCTTGTGAA
miR-99b	CACCCGTAGAACCGACCTTGC
miR-301	CGCAGTGCAATAGTATTGTCAAAGC
miR-26a	CGGTTCAAGTAATCCAGGATAGGCT
miR-26b	CGCGTTCAAGTAATTCAGGATAGGTA
miR-21	CGCGTAGCTTATCAGACTGATGTTG
miR-29a	CGTAGCACCATCTGAAATCGGTTA
miR-29b	CGTAGCACCATTTGAAATCAGTGTTA
miR-125b	TCCCTGAGACCCTAACTTGTGAAAA
miR-320a	AAAAGCGGGGAGAGGGCG
miR-140	CAGTGGTTTTACCCTATGGTAGAAA

2.2.5. Prediction and annotation of known miRNA targets

Targetscan (Lewis et al., 2005) software was used for prediction and annotation of known miRNA targets in the reference species.

2.2.6. Novel miRNA prediction

To avoid repeated prediction and to reduce the amount of calculations, we then searched against the genome and combined candidate unique reads whose distance in the reference genome was less than 100 bp. The 100 nt upstream and downstream sequences of the unique reads were included for secondary structure analysis. RNAfold (Hofacker, 2003) software was used to find inverted repeats. Unique reads in the inverted repeats were evaluated by miReap and mirCheck (Jones-Rhoades and Bartel, 2004) using modified parameters.

2.2.7. Prediction of miRNA targets of MEV and its specific receptor

RNAhybrid (Krueger and Rehmsmeier, 2006) was used to predict miRNA targets of MEV and its specific receptor following the rules of no mismatch and G/U complementarity in miRNA seed sequences.

2.3. Quantitative RT-PCR (qRT-PCR) of miRNAs

Total RNAs of the two samples were extracted and quantitated as described above, then polyadenylated using polyA polymerase (NEB) and reverse-transcribed using adapter primers with MLV reverse transcriptase (TaKaRa) (Yang et al., 2010). Selected miRNAs were subjected to qRT-PCR using Fast SYBR Green Mix (CWBIO) with ABI ViiA7. U6 small RNA was used as an internal control. Three independent biological replicates were conducted. All primers used are listed in Table 1.

Table 2
Small RNA signatures that match various RNAs.

Type	C3	Percent of total reads	V3	Percent of total reads
Total reads	6591749	100%	7553048	100%
Low quality	71327	1.08%	95029	1.26%
Adaptor3 null	4642	0.07%	2886	0.04%
Insert null	154726	2.37%	60710	0.81%
5′ adaptor contaminants	2605	0.04%	3151	0.04%
Size < 18 nt	55872	0.86%	237390	3.18%
polyA	1151	0.02%	182	0.00%
Clean.txt, size > = 18 nt	6301426	96.64%	7153700	95.92%

C3, uninfected F81 cells; V3, MEV-infected F81 cells.