



IDENTIFICATION OF A NOVEL MARINE FISH VIRUS, SINGAPORE GROUPEL IRIODOVIRUS-ENCODED MICRORNAS EXPRESSED IN GROUPEL CELLS BY SOLEXA SEQUENCING

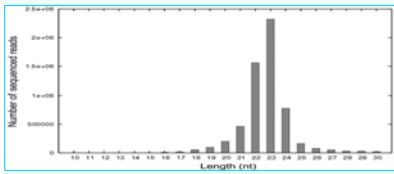
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1. Introduction

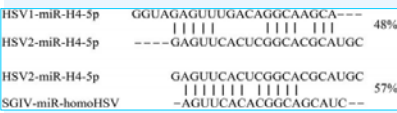
MicroRNAs (miRNAs) are ubiquitous non-coding RNAs that regulate gene expression at the post-transcriptional level. An increasing number of studies have revealed that viruses can also encode miRNAs, which are proposed to be involved in viral replication, persistence, and angiogenesis. Singapore grouper iridovirus (SGIV) is a pathogenic iridovirus that has severely affected grouper aquaculture in China and Southeast Asia. To determine whether SGIV encoded miRNAs during infection, a small RNA library derived from SGIV-infected grouper (GP) cells was constructed and sequenced by Illumina/Solexa deep-sequencing technology. We recovered 6,802,977 usable reads, of which 34,400 represented small RNA sequences encoded by SGIV. Sixteen novel SGIV-encoded miRNAs were identified by a computational pipeline, including a miRNA that shared a similar sequence to herpesvirus miRNA HSV2-miR-H4-5p, which suggests miRNAs are conserved in far related viruses. Generally, these 16 miRNAs are dispersed throughout the SGIV genome, whereas three are located within the ORF057L region. Some SGIV-encoded miRNAs showed marked sequence and length heterogeneity at their 3' and/or 5' end. Expression levels and potential biological activities of these viral miRNAs were examined by stem-loop quantitative RT-PCR and luciferase reporter assay, respectively, and 11 of these viral miRNAs were present and functional in SGIV-infected GP cells.

2. Results

2.1 Size distribution and abundance of sequenced small RNAs from SGIV-infected GP cells



2.2 Comparison of SGIV-miR-homoHSV and conserved HSV2-H4-2-5p.

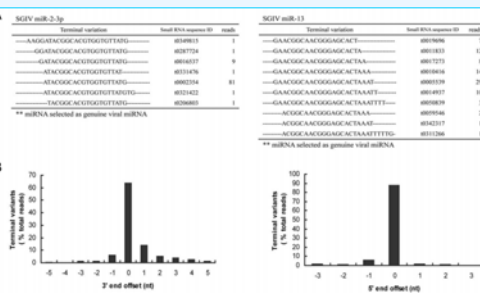


2.3 Sequence and genomic position of SGIV miRNAs.

SGIV miRNA	Sequence (5'-3')	Length (nt)	No. of reads	Genomic position*
miR-1.3p	(AATTATGAGTGAAGAGCCGAGGATG)	20-25	24	6201-6238
miR-1.4p	(TATGAGGAGCCGCTCAGTATTTT)	20-21	2	6199-6199
miR-2	(GAGGATGACGCGGCGGCTATATATG)	19-24	65	1929-1929
miR-3	(AAGCTGATGCTGCTGAGGAGCA)	20-22	7	4579-4812
miR-4	(GAGAGAGGCTATGAGGAGCA)	22	8	21173-21174
miR-5	(AATACAGCCGCTCTTATCTATCT)	19-23	97	2819-3421
miR-6	(AAGTGGAGGAGGAGGAGGAGG)	19-21	23	5149-5178
miR-7	(GAGGCTCATGAGGAGGAGGAGG)	20-24	7	5302-5385
miR-8	(TAAAGAGAGGAGGAGGAGGAGG)	18-24	36	6419-6419
miR-9	(TATACAGGAGGAGGAGGAGGAGG)	20-25	22	11918-11943
miR-10	(GAGGAGGAGGAGGAGGAGGAGG)	18-23	22	13228-13286
miR-11	(TATACAGGAGGAGGAGGAGGAGG)	20-22	11	13909-13932
miR-12	(CTACAGGAGGAGGAGGAGGAGG)	20-23	9	15141-15143
miR-13	(GATACAGGAGGAGGAGGAGGAGG)	19-26	67	16917-16934
miR-14	(AATACAGGAGGAGGAGGAGGAGG)	20-21	8	18796-18796
miR-homoHSV	(AGTTCACAGGAGGAGGAGGAGG)	18	4	11123-11940

SGIV miRNA sequences, length, number of reads and genomic positions are indicated.
*Sequence variations are indicated by asterisk in parentheses.
*Genomic positions are provided based on the published SGIV genome sequence (GenBank accession no. NC_205545).
*Complementary strand of the published SGIV genome sequence.

2.6 5' and 3' ends sequence variation of SGIV miRNAs recovered from deep sequencing.

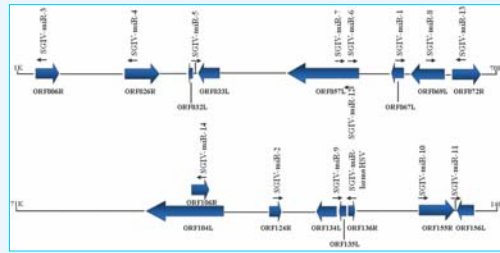


2.3 Distribution of small RNAs from GP cells infected with SGIV.

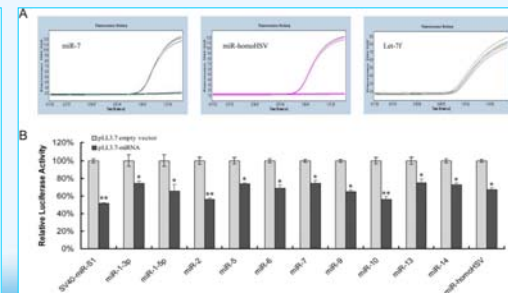
Small RNA class	Number of reads
Total high quality reads	5,884,103
Match with zebrafish genome	3,717,379
Conserved miRNA ^a	3,457,140
rRNAetc ^b	134,305
Repeat ^c	4,585
Match with SGIV genome	34,400
Unannotation ^d	2,132,324

^aIncludes 138 conserved miRNAs between zebrafish and grouper fish.
^bIncludes rRNA, tRNA, snRNA and snoRNA.
^cRepresents repeat-associated small RNAs.
^dIndicates sequences that do not match to SGIV or zebrafish genome.

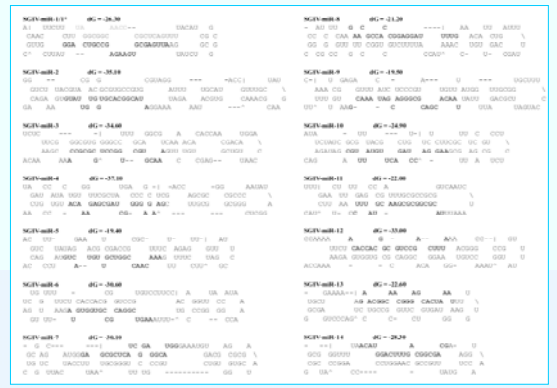
2.7 Schematic diagram of genomic location of miRNA precursors encoded by SGIV.



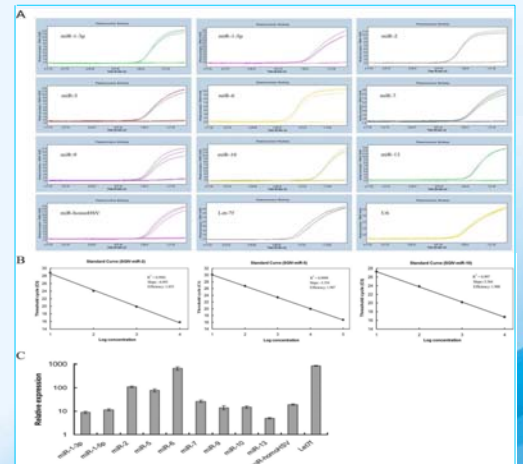
2.9 Biological activity of SGIV miRNAs.



2.4 Predicted stem-loop secondary structures of SGIV-encoded candidate miRNAs.



2.8 Stem-loop quantitative RT-PCR analysis of the SGIV miRNAs expression levels in SGIV-infected GP cells.



Our study provided a genome-wide view of miRNA production for iridoviruses and identified 16 novel viral miRNAs. To the best of our knowledge, this is the first experimental demonstration of miRNAs encoded by aquatic animal viruses. The results provide a useful resource for further in-depth studies on SGIV infection and iridovirus pathogenesis.

3. Conclusion