Identification and characterization of microRNAs from the tube foot in the sea urchin *Strongylocentrotus intermedius*

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**Abstract**

MicroRNAs (miRNAs) play critical roles in regulating many bio-processes of eukaryotes. The sea urchin *Strongylocentrotus intermedius* (an important fishery resource) is of great economic importance in Japan, North Korea, Russia, and China. In the current study, miRNAs of tube foot in *S. intermedius* were firstly identified and characterized. Data in this study can provide more genomic information for the further understanding of the complex regulation network in sea urchins and present a new way for monitoring the health status of cultured sea urchins.

**Keyword:** Genetics

**1. Introduction**

MicroRNAs (miRNAs) are short endogenous non-coding RNAs, with lengths of about 20–25 nucleotides (nt) (Chen et al., 2016). It has been well documented that miRNAs play vital roles in many physiological and biochemical processes of
eukaryotes (Wei et al., 2014). MiRNAs are also involved in host immune and stress response in eukaryotes, via regulating the expression of their target genes post-transcriptionally (Achkar et al., 2016). As for marine organisms, miRNAs have been identified from many species such as fish (Chen et al., 2017), crustaceans (Zhou et al., 2015), echinoderms (Wang et al., 2014; Mi et al., 2014), shellfish (Picone et al., 2016), and cephalochordates (Liao et al., 2017).

The sea urchin *Strongylocentrotus intermedius* is naturally distributed in northern regions of the Pacific coastal waters, the Sea of Japan, and Korean waters (Lawrence, 2013). In 1989, *S. intermedius* was transplanted from Japan waters by Dalian Ocean University, and it has become one of the most important cultured sea urchin species to date. In China, it is widely cultivated along the coastal areas of Liaoning and Shandong Provinces (Chang et al., 2012). According to China fishery Statistical Yearbook (2015), the annual aquaculture output of the sea urchin *S. intermedius* was 6.79 kilotons in 2014, as a result of the large demand for its gonad which is a highly valuable domestic and export product (Ministry of Agriculture of the People’s Republic of China, 2015). However, with the expansion of *S. intermedius* aquaculture and the continuous deterioration of its culture environment, it has been prominent to increase the disease resistance of *S. intermedius* in recent years (Wang et al., 2013).

Tube foot is an important organ for sea urchins. It functions in sensory, movement, attachment, and responding to environmental changes (Kabat-Zinn and Singer, 1981). As tube feet can be sampled non-destructively *in vivo*, the health status of sea urchins can be monitored at any time by tube feet sampling. To date, many genes and proteins of tube feet related to the functions mentioned above have been identified and characterized. However, the information of miRNAs of tube feet in sea urchins is still lacking.

In this study, miRNAs of tube feet in *S. intermedius* were identified and characterized by next-generation high-throughput sequencing techniques. Data observed here can increase our knowledge of tube foot miRNAs in sea urchins. It can also provide information for the further understanding of the complex regulation network in sea urchins when coping with different conditions.

2. Materials and methods

2.1. Sea urchin tube feet sampling

Fifteen healthy *S. intermedius* (average test diameter = 27.89 ± 1.14 mm) provided by the Key Laboratory of Mariculture & Stock Enhancement in North China’s Sea were randomly grouped into three groups (five each group) as replicates (SI1, SI2, SI3). The sample tubes were immediately transferred to cryovials and stored at -80 °C.
SI3) in this study. Tube feet collected from each individual within each group were pooled.

2.2. RNA extraction, RNA library construction, and sequencing

Total RNA extraction, small RNA library construction, Illumina sequencing, and transcriptome assembly were performed for each replicated pool as described by Zhong et al. (2015). An equal mixture of the tube foot RNA extracted from five individuals within each replicate was used to build the small RNA library. Raw reads were processed for the evaluation of sequencing quality, the removing of low quality reads and adaptor sequences, and the calculation of the length distribution of small RNA reads (Xu et al., 2015).

2.3. MiRNA identification

The remaining clean reads were aligned against known pre-miRNAs in miRbase 21.0 (http://www.mirbase.org/) to identify the conserved miRNAs. Only those small RNAs with their mature and precursor sequences perfectly matched to the known sea urchin miRNAs were considered to be conserved miRNAs. Two software, miREvo (Wen et al., 2012) and miRDeep2 (Friedlander et al., 2011), were used to predict novel miRNAs through exploring the secondary structure, the Dicer cleavage site, and the minimum free energy of the small RNA tags that were not annotated in previous steps. At the same time, custom scripts were applied to obtain the identified miRNA counts. Base bias with certain length on the first position and on each position of all the identified miRNAs were also obtained in this step.

3. Results

3.1. Data description

As shown in the results, an average of 8911741.67 raw reads (raw data) were obtained after sequencing on an Illumina Hiseq 2500 platform (Novogene Bioinformatics Technology Co., Ltd., Beijing, China), and an average of 7083236.67 clean reads (clean data) were then filtered from the raw data (Table 1). An average of 518539.67 unique sequences were observed from clean data, as candidates for miRNA analysis. All unique sequences were aligned against the known miRNAs in miRbase (v21.0, http://www.mirbase.org/) by BLAST (Basic Local Alignment Search Tool).

3.2. Data deposition

All the sequencing clean reads were deposited in the Short Read Archive (SRA) database (http://www.ncbi.nlm.nih.gov/sra/), which are retrievable under the
Table 1. Summary of the miRNA transcriptome sequencing of the tube foot in *S. intermedius*. SI1, SI2 and SI3 are replicates.

| Library                        | Count of reads       | Mean % of total | Mean       |
|--------------------------------|----------------------|-----------------|------------|
|                                | SI1      | SI2      | SI3      | SI1      | SI2      | SI3      | SI1      | SI2      | SI3      | SI1      | SI2      | SI3      |
| Raw reads                      | 9476098.00 | 8140985.00 | 9118142.00 | 8911741.67 | 100      | 100      | 100      | 100      | 100      | 100      | 100      | 100      |
| N% > 10%                       | 1.00     | 39.00    | 33.00    | 24.33     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     |
| low quality                    | 44565.00 | 27825.00 | 35480.00 | 35956.67 | 0.47     | 0.34     | 0.39     | 0.40     | 0.40     | 0.40     | 0.40     | 0.40     |
| 5_adapter_contamine            | 7469.00  | 5007.00  | 1526.00  | 4667.33  | 0.08     | 0.06     | 0.02     | 0.05     | 0.05     | 0.05     | 0.05     | 0.05     |
| 3_adapter_null or insert_null  | 2271046.00 | 1112001.00 | 1959048.00 | 1783698.33 | 23.97    | 13.77    | 21.49    | 19.74    | 19.74    | 19.74    | 19.74    | 19.74    |
| with ployA/T/G/C               | 2898.00  | 5484.00  | 4093.00  | 4158.33  | 0.03     | 0.07     | 0.04     | 0.05     | 0.05     | 0.05     | 0.05     | 0.05     |
| rRNA                           | 1950339.00 | 1498864.00 | 2238924.00 | 1896042.33 | 0.21     | 0.18     | 0.25     | 0.21     | 0.21     | 0.21     | 0.21     | 0.21     |
| known_miRNA                    | 4112.00  | 5264.00  | 3322.00  | 4232.67  | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     |
| tRNA                           | 0.00     | 1.00     | 1.00     | 0.67     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     |
| snRNA                          | 104.00   | 111.00   | 98.00    | 104.33   | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     |
| snoRNA                         | 817.00   | 577.00   | 504.00   | 632.67   | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     |
| novel_miRNA                    | 116695.00 | 58033.00 | 42348.00 | 72358.67 | 0.01     | 0.01     | 0.00     | 0.01     | 0.01     | 0.01     | 0.01     | 0.01     |
| other                          | 1633190.00 | 2532754.00 | 2459310.00 | 2208418.00 | 0.17     | 0.31     | 0.27     | 0.25     | 0.25     | 0.25     | 0.25     | 0.25     |
| clean reads                    | 7150119.00 | 6981629.00 | 7117962.00 | 7083236.67 | 75.45    | 85.06    | 78.06    | 79.52    | 79.52    | 79.52    | 79.52    | 79.52    |
| 18nt                           | 22825.00 | 21287.00 | 33469.00 | 25860.33 | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     | 0.00     |
| 19nt                           | 52200.00 | 65623.00 | 114338.00 | 77387.00 | 0.01     | 0.01     | 0.01     | 0.01     | 0.01     | 0.01     | 0.01     | 0.01     |
| 20nt                           | 189334.00 | 206819.00 | 333449.00 | 243200.67 | 0.02     | 0.03     | 0.04     | 0.03     | 0.03     | 0.03     | 0.03     | 0.03     |

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Table 1. (Continued)

| Library | Count of reads | Mean | % of total | Mean |
|---------|----------------|------|------------|------|
|         | SI1            | SI2  | SI3        | SI1  |
| 21nt    | 572291.00      | 521369.00 | 751698.00  | 615119.33 |
| 22nt    | 3209445.00     | 2325028.00 | 2315233.00  | 2616568.67 |
| 23nt    | 1471068.00     | 947966.00  | 846541.00  | 1088525.00 |
| 24nt    | 170896.00      | 119441.00  | 114960.00 | 135099.00 |
| 25nt    | 82657.00       | 86359.00  | 66465.00  | 78493.67 |
| 26nt    | 89247.00       | 151105.00 | 112963.00  | 117771.67 |
| 27nt    | 151490.00      | 242906.00 | 191590.00  | 195328.67 |
| 28nt    | 528928.00      | 1301596.00 | 1283661.00 | 1038061.67 |
| 29nt    | 314882.00      | 548439.00 | 530376.00  | 464565.67 |
| 30nt    | 108349.00      | 171554.00 | 168642.00  | 149515.00 |
| 31nt    | 45733.00       | 56481.00  | 43980.00  | 48731.33 |
| 32nt    | 24132.00       | 35199.00  | 24415.00  | 27915.33 |
| 33nt    | 13544.00       | 24251.00  | 17755.00  | 18516.67 |
| 34nt    | 10175.00       | 18322.00  | 13640.00  | 14045.67 |
| 35nt    | 6635.00        | 13880.00  | 10368.00  | 10294.33 |
4. Discussion

As shown in the results, miRNAs with a length of 22nt had the highest percentage of all identified miRNAs in the three replicates (Table 1). This is consistent with a previous study showing that miRNAs with a length of 22nt had the highest percentage in *Andrias davidianus* (Huang et al., 2017a,b). In order to search for the miRNAs expressed in all three replicates, miRNA expression levels of each pooled sample were estimated by TPM (transcript per million) through the criteria of Zhou et al. (2010). A total of forty-one known miRNAs and twenty novel miRNAs (TPM > 0) were identified from the three replicates (Table 2 and Table 3). The three most abundantly expressed known miRNAs were spu-miR-184, spu-miR-7, and spu-miR-1. Wang et al. found that miR-184 and miR-1 were two of the most expressed known miRNAs in the tube foot of healthy sea cucumber *Apostichopus japonicas* (Wang et al., 2014). Taken both results together, we hypothesis that the expression trends of miR-184 and miR-1 were consistent in echinoderms. GO (Gene Ontology) analysis (http://www.geneontology.org/) showed that the identified miRNAs might regulate multiple genes involved in cellular components, molecular functions, and several bio-processes such as metabolic process, response to stimulus, and catalytic activity (Figs. 1-b and 2-b). This result could facilitate further studies on the specific roles played by miRNAs in sea urchins. Moreover, it is worthwhile to note that the number of conserved miRNAs in tube feet of the sea urchin *S. intermedius* was less than that in tube feet of the sea cucumber *Apostichopus japonicas*, while the number of novel miRNAs in tube feet of *S. intermedius* was more than that in tube feet of *A. japonicas* (Wang et al., 2014). This observation indicates that miRNA expression profiles might vary among species. Many studies have documented that uracil (U) is the most common base as the first nucleotide located at 5' end of miRNA (Greagg et al., 1999). A similar result was observed in the current study. An average of 78.56% of known miRNAs and an average of 84.23% of novel miRNAs had a relatively higher percentage of U at the first position in the tube foot of *S. intermedius* (Figs. 1-a and 2-a). The “seed region” (defined as the 2nd to the 8th nucleotides of miRNAs) has been demonstrated to be responsible for targeting mRNAs for gene regulation (Huang et al., 2017a,b). The strong bias of U at the 1st and 9th nucleotides might regulate miRNA-mRNA interaction through flanking the edges of the “seed region” (Zhang et al., 2009). Compared to the results from Mi et al. that 58.3% of known miRNAs from gonads of *Strongylocentrotus nudus* tend to use U as the first base (Mi et al., 2014), conserved miRNAs in the tube foot of *S. intermedius* exhibited a relatively stronger bias of U at the first position. Therefore, we postulate that there are species-specific and tissue-specific variabilities in conserved miRNAs.
Table 2. Known miRNAs identification from the tube foot of *S. intermedius*.

| Known-miRNA    | Sequences (5'-3') | Length | Mean readcount | Mean TPM  |
|----------------|-------------------|--------|----------------|----------|
| spu-miR-92d    | UAUUGCACUUACCCCGGCUG | 20     | 122.67         | 65.22    |
| spu-miR-124    | UAGGCAACCGCGUGAAGGCCA | 21     | 10.00          | 5.28     |
| spu-miR-96     | UUGGCCACUAGCAAUUUGC | 21     | 21.67          | 11.59    |
| spu-miR-2013   | UGCAGCAUGAUAGUAGUGGUGU | 21    | 106.00        | 55.23    |
| spu-miR-92e    | UAUUGCAUUCACCCGGCUUA | 21     | 161.33         | 82.26    |
| spu-miR-31     | AGGCAAGAUUGUAGCAGCU | 21     | 1887.67        | 9951.81  |
| spu-miR-2010   | UUAUCUGUAUGUCAGCCCUU | 22     | 2.67           | 1.28     |
| spu-miR-252b   | CUAAGAUGUAGUAGCCGCAUC | 22    | 2.67           | 1.27     |
| spu-miR-183    | UAUUGGCAUAAGAAUUCAGUG | 22    | 3.00           | 1.42     |
| spu-miR-210    | UUGUGCGUGCGACAGCAGCAGU | 22   | 5.33          | 2.78     |
| spu-miR-137    | UAUUGCUUAGAUAACACGUGA | 22   | 11.33         | 6.22     |
| spu-miR-92b-3p | UAUUGCACUUGUCCCGGUCAU | 22   | 17.33         | 9.21     |
| spu-miR-2001   | AUGUGACCGAUAAUGGCGAU | 22   | 38.33         | 20.92    |
| spu-miR-278-5p | UGGAAUGAAACGCCGCCCAACU | 22 | 55.33      | 28.64    |
| spu-miR-9-3p   | AUAAAGCUAGGUUACCAAGAU | 22   | 60.00         | 31.03    |
| spu-miR-278-3p | UCGGGUGGACUUIUCGUAGCU | 22   | 61.00         | 30.88    |
| spu-miR-4852   | AAUUCUUAUCUAUUGGCGCUA | 22   | 78.67         | 41.27    |
| spu-miR-2011   | ACCAAGGUGUAGUAGUAGAC | 22   | 500.00        | 248.08   |
| spu-miR-125-3p | ACACGGUUGGUAUCUGAGGAU | 22  | 631.00        | 325.71   |
| spu-miR-9-5p   | UCUUUGGUUAAUCUGCUAGU | 22   | 802.00        | 410.52   |
| spu-miR-153-3p | UUGCAUAGUCACAAAGUGAU | 22   | 1706.33       | 906.22   |
| spu-miR-200-5p | CAUCUAGCAGGCAUCUGAGA | 22   | 2654.67       | 1362.21  |
| spu-miR-4847   | UAUUGAUGCGCUGCUGCGUGC | 22   | 3506.00       | 1902.39  |
| spu-let-7      | UGGAGGUAGUAGGUAUAAGUU | 22   | 6961.00       | 3571.52  |
| spu-miR-375    | UUGUUCGUUCGCUCGCUCGCAA | 22 | 10442.00  | 5430.47  |
| spu-miR-2004   | UCACACACACACACACAGGAAAGU | 22  | 12090.67  | 6153.14  |
| spu-miR-29a    | AAGCAGCAGUGUAAUAUCAGAC | 22   | 13320.33     | 7146.20  |
| spu-miR-2012   | UAGUACUGGCCAUUGGCAACUG | 22   | 20244.33     | 10439.92 |
| spu-miR-125-5p | UCCCGAGACCUACUACUGUGA | 22   | 23805.00     | 12701.23 |
| spu-miR-34     | CGGCAGUGUAUUGAUGCUGUGU | 22   | 29303.33     | 15381.47 |
| spu-miR-1      | UGGAAUAGUAGGAAAGUAUGUA | 22   | 266969.00    | 135440.97 |
| spu-miR-184    | UGGAGGAGAAGCUAGUAGGCG | 22   | 947961.00    | 479823.98 |
| spu-miR-2003-3p| CAGGUUAUGGCUUUGGUGAUA | 23  | 1.00          | 0.52     |
| spu-miR-92a    | UAUUGCAACUUGCCCGCUACU | 23   | 17.33         | 9.21     |
| spu-miR-10     | AACCUCUGUAACCCGAAUUUGGUG | 23  | 70.00        | 39.08    |
| spu-miR-2007   | UAUUCUGGACGAGUAACUGGUA | 23   | 249.33        | 131.06   |
| spu-miR-71     | UGAAAGACAUUGGGAUGAGAU | 23   | 2640.00       | 1423.26  |
| spu-miR-2002-3p| UGAAUACACUGCGUGUUUUAU | 23  | 2793.33       | 1439.96  |

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when regulating target mRNAs in echinoderms. We also found that novel miRNAs with lengths of 24nt and 26nt exhibited a bias about 40.15%–50.00% of adenine (A) at the first position, which may need further research to study and clarify.

In conclusion, an overview of miRNAs in the tube foot of sea urchin *S. intermedius* is provided in this preliminary study. Observations in this study increase the knowledge of non-coding RNAs in sea urchins and provide a new way for monitoring the health status of cultured sea urchins.

Table 2. (Continued)

| Known-miRNA | Sequences (5’-3’) | Length | Mean readcount | Mean TPM |
|-------------|-------------------|--------|----------------|---------|
| spu-miR-200-3p | UAAUACUGUCUGUGAUAGUGU | 23 | 43303.00 | 22430.70 |
| spu-miR-7 | UGGAAGACUAUGUAAUUGUGU | 23 | 483537.67 | 245439.18 |
| spu-miR-182 | UUGGCAUAUGUAAGAAUCACACU | 25 | 26.67 | 13.18 |

miRNA expression levels were estimated by TPM (transcript per million) through the Normalization formula: Normalized expression = mapped read count/Total reads*1000000.

Table 3. Novel miRNAs identification from the tube foot of *S. intermedius*.

| Novel_miRNA | Sequences (5’-3’) | Length | Mean readcount | Mean TPM |
|-------------|-------------------|--------|----------------|---------|
| novel_137 | uuauacacugugucaucca | 20 | 5.00 | 2.43 |
| novel_98 | uguuauauguguaaacaaggg | 21 | 18.67 | 10.03 |
| novel_181 | aauucggucuuaagaacaga | 21 | 41.33 | 20.34 |
| novel_50 | aataucugccuccuuaacacc | 22 | 2.00 | 1.04 |
| novel_79 | uccguuucguagacuagcacc | 22 | 2.33 | 1.12 |
| novel_121 | uuuucguucuuuccuguguu | 22 | 2.67 | 1.35 |
| novel_147 | augggcucuacaacacauau | 22 | 3.00 | 1.42 |
| novel_87 | uuuccacaagugucagguagug | 22 | 3.33 | 1.66 |
| novel_45 | aauuuugucgccgcuagcuag | 22 | 4.67 | 2.41 |
| novel_39 | auggcgcgcgcguagguaguag | 22 | 6.67 | 3.80 |
| novel_194 | ucgacacucuccucacacgcug | 22 | 11.67 | 6.67 |
| novel_70 | uugacauucccaaggcgugacg | 22 | 13.33 | 6.91 |
| novel_134 | uggugucucuucugcaagcuau | 22 | 28.67 | 15.78 |
| novel_20 | uuucacacugcuagacagg | 22 | 84.67 | 44.86 |
| novel_202 | cugauugucuacaaacggg | 22 | 127.00 | 63.95 |
| novel_7 | uggguuguugguuauagguag | 22 | 36356.00 | 18847.28 |
| novel_118 | uuuguucgcuucgcuacuau | 23 | 327.33 | 169.86 |
| novel_8 | uuucugucuucuugguauag | 23 | 35236.00 | 18243.18 |
| novel_163 | acaaugucugacgagguacacu | 24 | 6.33 | 3.16 |
| novel_113 | auggacacugcugcucacucucca | 24 | 9.67 | 5.54 |

miRNA expression levels were estimated by TPM (transcript per million) through the Normalization formula: Normalized expression = mapped read count/Total reads*1000000.
Fig. 1. First position nucleotide percentage and GO terms for predicted target genes of known miRNAs analyses of the tube foot in *S. intermedius*. a. Analysis of the nucleotide percentage at the first position of known miRNAs. b. GO terms for predicted target genes of known miRNAs of the tube foot in *S. intermedius*.

Fig. 2. First position nucleotide percentage and GO terms for predicted target genes of novel miRNAs analyses of the tube foot in *S. intermedius*. a. Analysis of the nucleotide percentage at the first position of novel miRNAs. b. GO terms for predicted target genes of novel miRNAs of the tube foot in *S. intermedius*.
Declarations

Author contribution statement

Yaoyao Zhan: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yingying Li: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dongyao Cui, Jingxian Sun: Performed the experiments.

Qiantong Pei: Analyzed and interpreted the data; Wrote the paper.

Weijie Zhang: Analyzed and interpreted the data.

Yaqing Chang: Conceived and designed the experiments.

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Competing interest statement

All authors have no conflict of interest.

Additional information

Data associated with this study (all the sequencing clean reads) has been deposited at the Short Read Archive (SRA) database (http://www.ncbi.nlm.nih.gov/sra/) under the accession numbers SRR6251260 (SI1), SRR6251258 (SI2), SRR6251259 (SI3).

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