Computational identification and characterization of miRNAs and their target genes from five cyprinidae fishes

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Received 8 November 2014; revised 2 May 2015; accepted 6 May 2015
Available online 14 May 2015

Abstract MicroRNAs (miRNAs) are a kind of small single-strand RNA molecules with lengths of 18–25 nt, which do not encode any proteins. They play an essential role in gene expression regulation by binding to their target genes, leading to translational repression or transcript degradation. In this study, 23 miRNAs were predicted from five cyprinidae fishes by using a bioinformatics-based gene search based on blasting ESTs and GSS in NCBI, of which 21 miRNA genes have not been previously reported. To prove their validity, five of the computationally predicted miRNAs were verified by RTPCR, their transcripts were successfully detected, and, 46 potential target genes for these miRNAs were predicted, most target genes encode transcription factors, they are involved in signal transduction, metabolism and development processes.

1. Introduction

MicroRNAs (miRNAs) are endogenous small non-coding RNAs with lengths ranging from ~18 to 25 nucleotides in size, which negatively regulate gene expression by directing mRNA cleavage or interfering with translation (Ambros, 2004; Bartel, 2004; Kloosterman and Plasterk, 2006; Sun and Lai, 2013). In the nucleus, these small miRNA molecules exist as independent transcription units, which are transcribed into long primary transcripts (pri-miRNAs) by RNA polymerase II, and then cleaved to long self-complementary miRNA precursors (pre-miRNAs) (Almeida et al., 2011; Gu et al., 2012). Then, pre-miRNA is exported to the cytosol and cut into a short double-stranded RNA by the Dicer nuclease (Bartel, 2004; Song et al., 2012). Finally, the single-stranded mature miRNA is then selectively loaded into the RNA-induced silencing complex (RISC) that contains Argonaute family proteins where it regulates targets by either cleaving target miRNAs or repressing the translation process (Chua et al., 2009; Friedlander et al., 2014; Graves and Zeng, 2012;
Many studies have demonstrated that miRNAs have multiple roles in animal diverse biological processes, including organ development, cell proliferation and division, pathological processes, fat metabolism, hormone secretion, embryogenesis, neural development, apoptosis and so on (Bartel, 2009; Bhaskaran and Mohan, 2013; Kloosterman and Plasterk, 2006; Ladomery et al., 2011; Lucas and Raikhel, 2013; Naqvi et al., 2012). It is estimated that miRNAs as the key regulators comprise 1–5% of animal genes and regulate up to 30% of genes (Friedman et al., 2009; Hendrickson et al., 2009; John et al., 2004).

Cyprinidae fishes are an important aquaculture species around the world, for example *Cyprinus carpio*, *Carassius auratus*, and the four domestic fish (*Mylopharyngodon piceus*, *Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*), which occupies a prominent position in the world of freshwater aquaculture and serves as a major source of animal protein for millions of people especially in China and several other East-Asia countries (Gui and Zhou, 2010; Liao et al., 2007). Fish represent approximately half of all vertebrate species. Although thousands of miRNA genes have been reported in mammals, insects, worms, plants, and viruses, but research on cyprinid fishes miRNAs was seldom reported. According to the latest miRNA Registry Database (http://www.mirbase.org/; released on 21 June, 2014), there are only nine fish miRNA in repository until now. miRNAs identified in fish have been limited to *C. carpio*, *Danio rerio*, *Hippoglossus hippoglossus*, *Fugu rubripes*, *Ictalurus punctatus*, *Oryzias latipes*, *Tetraodon nigroviridis*, *Salmo salar* and *Paralichthys olivaceus*. miRNAs can be identified through the cloning method, high-throughput sequencing method and computational approaches (Baev et al., 2009; de Souza Gomes et al., 2013; Qi et al., 2014; Wang et al., 2013; Wu et al., 2010). Compared to the experimental methods, computational approaches based on highly conserved miRNA in animals and plants have been proved to be faster, more affordable and more effective (Chaudhuri and Chatterjee, 2007; Hou et al., 2008; Li et al., 2010). Some predicted miRNA based computational approaches cannot be detected by direct cloning, particularly those miRNAs which were in low abundance, but computational approaches apply not only to the species with complete genomic information but also to those whose complete genome sequences are unavailable but have rich expressed sequence tag (EST) sequences and Genomic Survey Sequences (GSS).

In this study, we used all reported animal miRNAs deposited in the miRNA database (miRBase) to blast search the five cyprinid fishes miRNAs homologs in the ESTs and GSSs from the NCBI GenBank database, which are *C. carpio*, *M. anguillicaudatus*, *C. auratus*, *M. amblycephala* and *C. alburnus*, respectively. A total of 23 potential miRNAs was predicted and their characteristics were investigated. The 21miRNAs were newly discovered in five different cyprinid fishes. Five miRNA were validated by Stem-loop RT-PCR. In addition, 46 potential targets for the predicted miRNAs were identified. This research will provide useful information for miRNA research in cyprinid fishes and other aquaculture species, and for future elucidation of regulatory roles of miRNAs in growth, organ development, metabolism, and other biological processes.
Table 1  The 23 newly identified miRNAs from five cyprinidae fishes.

| miRNAs name | miRNA homologs | Gene source | Mature sequence (5' to 3') | Side | NM (nt) | Strand | LP (nt) | A + U (%) | MFEs  |
|--------------|----------------|-------------|-----------------------------|------|--------|--------|--------|-----------|--------|
| ccr-miR-6732 | hsa-miR-6732   | JZ508372(EST)| CAGAAGUGGCCAGGCUGGCC      | 3'   | 3      | Minus  | 84     | 38.1      | −38.9  |
| ccr-miR-430a | ccr-miR-430   | HR561547(GSS)| UAAUGCUAUUUGUGGUGGUGA    | 3'   | 0      | Plus   | 80     | 57.5      | −25.9  |
| ccr-miR-430b | dre-miR-430b   | HN151353(GSS)| AAAGUGCUAUCAAGUUUGGGUAA  | 3'   | 1      | Minus  | 78     | 61.5      | −25.1  |
| ccr-miR-430c-3p| dre-miR-430c-3p| HR561547(GSS)| UAAUGCUUCUUUUGGGGUGA    | 3'   | 0      | Plus   | 92     | 64.1      | −35.6  |
| ccr-miR-365  | ccr-miR-365    | HR561450(GSS)| AAACUUUUUGGGGCAAUUA     | 3'   | 4      | Plus   | 119    | 68.9      | −21.6  |
| ccr-miR-2783 | bmo-miR-2783  | HR551227(GSS)| UAAUCAGGGUGUGGUGGGGA    | 3'   | 4      | Plus   | 96     | 60.4      | −30.2  |
| cau-miR-3198 | hsa-miR-3198  | GE468290(EST)| UUGGAUUCCUGGGGAAUGGAG  | 5'   | 1      | Minus  | 92     | 43.4      | −34.7  |
| cau-miR-1814b| bta-miR-1814b | FG394205(EST)| CUAUUGGUUAGUUGUUUUU    | 3'   | 3      | Plus   | 129    | 67.4      | −16.3  |
| cau-miR-2742 | bmo-miR-2742  | FG394388(EST)| UGUUCAUGGAUAUGGUUU      | 5'   | 1      | Minus  | 89     | 53.9      | −17.7  |
| cau-miR-149  | bta-miR-149   | AM403731(GSS)| UCUGGCUCCGUGCUUCAGCUUU | 3'   | 4      | Minus  | 136    | 45.6      | −56.4  |
| man-miR-4037 | hsa-miR-4703  | GAAD0101102(GSS)| CGGCAACAGUGGCAACAG    | 5'   | 3      | Plus   | 100    | 52.0      | −31.0  |
| man-miR-6751-3p | hsa-miR-6751-3p | GAAD01001061(GSS)| GCUGAGCCUCUCUCUCUCUC    | 3'   | 3      | Minus  | 72     | 52.3      | −17.6  |
| man-miR-7847-3p | hsa-miR-7847-3p | GAAD0101515(GSS)| GCUGAGGUGUCAAGAGGAGGC  | 3'   | 1      | Minus  | 148    | 39.2      | −58.9  |
| man-miR-142-3p | ccr-miR-142-3p | GAAD0109618(GSS)| GUAGUUGUUCAUACUAUGG    | 3'   | 0      | Minus  | 92     | 55.4      | −39.9  |
| man-miR-2452 | bta-miR-2452  | GAAD0100939(GSS)| CAGCGAUGUUGUUUCUUU     | 3'   | 3      | Plus   | 150    | 57.3      | −34.3  |
| man-miR-1603 | bta-miR-1603  | GAAD0102573(GSS)| CUGUGUUUUUGUUGUUUAU    | 3'   | 2      | Minus  | 108    | 65.7      | −17.6  |
| man-miR-2487 | bta-miR-2487  | GAAD01000444(GSS)| CUCUAAGGCGCGGGCGCGGCG  | 3'   | 0      | Minus  | 125    | 46.4      | −46.5  |
| man-miR-10a-5p | dre-miR-10a-5p | FJ746716(GSS)| AUACCCUCAGAUCGGAAUUUG  | 5'   | 3      | Minus  | 148    | 60.1      | −56.1  |
| man-miR-10a-3p | hsa-miR-10a-3p | FJ746716(GSS)| CAAUCUCUGUAUCGGAGAGAUA  | 3'   | 1      | Plus   | 110    | 60.0      | −40.5  |
| man-miR-2369 | bta-miR-2369  | GQ903705(GSS)| UAAUGUGUGUCUUUUCUCUG    | 3'   | 4      | Minus  | 78     | 57.7      | −17.1  |
| cal-miR-4483 | hsa-miR-4483  | FJ875089(GSS)| GGGUGUGUGUGUGGUGUUC      | 5'   | 1      | Plus   | 160    | 46.8      | −31.8  |
| cal-miR-6852 | hsa-miR-6852  | GU218201(GSS)| UUUUCUCUCUCUCUCACG      | 5'   | 1      | Minus  | 87     | 52.9      | −17.2  |
| cal-miR-5600-3p | cin-miR-5600-3p | KF111429(GSS)| UGUGGAAUGUUGUUUGUGUCUU | 3'   | 4      | Plus   | 136    | 54.4      | −32.9  |

NM, number of mismatch; LP, length of precursor; MFEs, minimal folding free energy (kcal/mol).
2. Materials and methods

2.1. miRNA reference sets

All known miRNA sequences in various animal species including fishes were obtained from miRBase (http://www.mirbase.org). To avoid the overlapping miRNAs, the repeat sequences of miRNAs within the above species were removed. The EST, GSS, and mRNA sequences of five cyprinidae fishes were obtained from the NCBI, which were used for miRNA prediction. The EST sequences of *C. carpio*, *M. anguillicaudatus*, and *C. auratus* were 50769, 22174 and 13937, respectively; the GSS sequences of *C. carpio*, *M. anguillicaudatus*, *C. auratus*, *M. amblycephala*, and *C. alburnus* were 72932, 12268, 4621, 809 and 236, respectively.

2.2. Computational prediction of miRNAs

Comparative software BLAST tool was downloaded from NCBI. BLASTN parameters were the same as those described in previous papers (Huang et al., 2010). Procedure of search for potential miRNAs was shown in Fig. 1. Five criteria used to distinguish miRNAs and pre-miRNAs from other kinds of RNAs were as follows: (1) predicted mature miRNAs were allowed to have only 0–4 nucleotide mismatches in sequence with all previously known animal mature miRNAs; (2) pre-miRNA sequence can fold into an appropriate hairpin secondary structure that contains the \( \text{C}2_{22} \) nt mature miRNA sequence within one arm of the hairpin structure; (3) miRNA precursors with secondary structures had higher negative minimal free energies (MFES) and minimal free energy index (MFEIs) than other different types of RNAs by RNA-fold prediction software; (4) miRNA had 30–70% contents of A + U by SVM (support vector machine) (Xu et al., 2008); and (5) no loop or break in miRNA sequences was allowed. If the sequence met all these criteria, it will be considered as a miRNA.

2.3. Stem-loop RT-PCR assay

To verify computational predictions, five miRNAs were randomly selected from the novel predicted miRNA by the stem-loop RT-PCR experiment method. Small RNA sequences were labeled with red capital letters.

Figure 2 Predicted stem-loop structures of newly identified precursor miRNAs from five cyprinidae fishes. The mature miRNAs were labeled with red capital letters.
the fish mixed tissues (skeletal muscle, brain, liver, and spleen) was extracted using an RNeasy Mini Kit (Qiagen), according to the supplier’s protocol. The cDNAs were synthesized from small RNAs using miRNA specific stem-loop RT primers according to criteria described previously (Chen et al., 2005; Mohammadi-Yeganeh et al., 2013; Varkonyi-Gasic and Hellens, 2011). The stem–loop RT primers and gene specific primers were listed in Table S1. 100 ng cDNA was used as template for the PCR. The PCR was programed as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 25 s and a final elongation step at 72°C for 7 min. The PCR products were separated through 2.5% (w/v) agarose gel. DNA fragments were directly subcloned into PMD18-T vector (Takara) and sequenced.

2.4. Phylogenetic analysis of the new miRNAs

Due to the conservation of miRNAs and their precursors, the precursor sequences of the novel and the known miRNAs in the same family were aligned by Clustal W, and then the maximum likelihood trees were constructed with MEGA 5.0, the neighbor-joining method with default bootstrap values was set, the phylogenetic tree illustrated the evolutionary relationships with other members of the same family (Larkin et al., 2007; Tamura et al., 2004, 2011). The results were saved.

2.5. Prediction of miRNA targets and their functions

It has been reported that the target genes of miRNAs could be predicted according to their complementarity with mature miRNA sequences (Carre et al., 2013; Grimson, 2010). In the present study, the target genes are predicted with the web-based computational software RNA hybrid program (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid) according to its operation manual (Rehmsmeier et al., 2004). The parameters were described as follows: P value cutoff of 0.05, target duplex free energy $\Delta G \leq -24$ kcal/mol. The criteria for the target gene identification were as follows: (1) four or fewer mismatched nucleotides at complementary sites between miRNA sequences and potential mRNA targets; (2) one mismatch allowed between position 2nd and 12th, but not at nucleotide positions 10th or 11th; (3) less than three additional mismatches between nucleotide positions 12–23, but no more than two continuous mismatches within this region.
**Figure 3**  Multiple sequence alignment analysis of pre-miR-142 (man-miR-142) family. Abbreviations: mmu, *Mus musculus*; has, *Homo sapiens*; rno, *Rattus norvegicus*; gga, *Gallus gallus*; dre, *Danio rerio*; fra, *Fugu rubripes*; tni, *Tetraodon nigroviridis*; xtr, *Xenopus tropicalis*; bta, *Bos Taurus*; mdo, *Monodelphis domestica*; oan, *Ornithorhynchus anatinus*; mml, *Macaca mulatta*; cfa, *Canis familiaris*; xla, *Xenopus laevis*; ptr, *Pan troglodytes*; eca, *Equus caballus*; ssc, *Sus scrofa*; cgr, *Cricetulus griseus*; ggo, *Gorilla gorilla*; ccr, *Cyprinus carpio*; aca, *Anolis carolinensis*; olu, *Oryzias latipes*; sha, *Sarcophilus harrisii*; cgr, *Cricetulus griseus*; ggo, *Gorilla gorilla*; eca, *Equus caballus*; ssa, *Salmo salar*; efu, *Eptesicus fuscus*; tch, *Tupaia chinensis*; oha, *Ophiophagus Hannah*; man, *Misgurnus anguillicaudatus*. Asterisks indicate conserved region.
3. Results and discussion

3.1. Identification of miRNAs

Sequence and structure homologies are the main theory behind the computer-based approach for miRNAs prediction. In this study, the similarity searches for miRNAs in the EST and GSS sequences yielded 62 matches, which were used for secondary structure prediction properties by RNA fold software prediction. Finally, some possible false sequences of pre-miRNAs were further eliminated by manual inspection. This resulted in 23 potential miRNAs. Among 6 miRNAs were identified in \textit{C. carpio}, 4 miRNAs were identified in \textit{C. auratus}, 7 miRNAs were identified in \textit{M. anguillicaudatus}, 3 miRNAs were identified in \textit{M. amblycephala}, and the rest 3 miRNAs were identified in \textit{C. alburnus} (Table 1). These newly predicted miRNAs were all first time reported except miRNA-365 and miRNA-430 family were previously identified (Yan et al., 2012; Zhu et al., 2012). The predicted miRNAs found belong to 21 miRNA families and every miRNA family has only one member, but miRNA-430 family has three members and miRNA-10a family has two members. The length of the predicted miRNAs was in the range from 72 nt to 160 nt, with an average of 109 nt. These sequences folded into a typical stem-loop structure, having the mature miRNA on the 5' arm end, or alternatively on the 3' arm end (Fig. 2). The hairpin loop secondary structures had a minimum folding free energy ranging from -58.9 kcal/mol to -16.3 kcal/mol. The new predicted miRNAs were also evaluated for A + U content, and results showed that the A + U contents ranged from 38.1% to 68.9% in miRNA precursors, which was consistent with previous studies on other animal (Ambros et al., 2003; Gong et al., 2010; Zhang et al., 2006; Zhou and Liu, 2010). These results showed that these predicted fishes miRNAs meet these strict screening criteria.

3.2. Conserved study and phylogenetic analyses

miRNAs always showed a conserved nature among the living organisms. Our study was based on the use of the pre-miRNAs rather than mature sequences in homology search. The conservation of mature miRNAs and their precursors provides the chance to investigate their evolutionary relationships. We chose one conserved pre-miRNA sequence from miRNA-142 family which was aligned by Clstw soft in the miRBase database. Results showed that pre-miRNA sequence from different
species have same conserved sequence in 5’arm and 3’arms (Fig. 3). These data suggest that miRNAs may present a conserved organization pattern among animals in very early evolution. Furthermore, the one big miRNA family miRNA-142 was selected for phylogenetic analyses. The phylogenetic tree analysis among the members of this family illustrated the evolutionary relationships of M. anguillicaudatus miRNA which is more closed to the S. salar, D. rerio, I. punctatus and T. nigroviridis species (Fig. 4).

3.3. Experimental verification of predicted miRNAs

The efficiency of the computational strategy was tested by biological experiments to validate the predicted miRNA genes. A total of 5 miRNAs were selected at random, of which miRNAs from different fishes: ccr-miR-430b, cau-miR-3198, man-miR-142-3p, mam-miR-10a-5p and cal-miR-4483 were subjected to the stem-loop RT-PCR for validation studies. The transcripts of 5 miRNA genes were successfully detected, demonstrating the expression of mature miRNAs (Fig. 5).

3.4. Prediction of potential targets of miRNAs

Researches have confirmed that miRNAs are mainly complementary to their target mRNAs in animals, which is different from miRNAs binding their targets by complete or nearly complete complementarity in plants (Martinez-Sanchez and Murphy, 2013; Trakooljul et al., 2010; Wang et al., 2013).

| miRNA       | Targeted protein                                | Target function          | Targeted genes       |
|-------------|-------------------------------------------------|---------------------------|----------------------|
| ccr-miR-6732| Toll-like receptor 2                            | Signal transduction       | FJ858800             |
|             | Putative delta-6 fatty acyl desaturase          | Metabolism                | AF309557             |
|             | HMG box transcription factor Sox9b              | Transcription factor      | AY874424             |
|             | ATP synthase                                    | Metabolism                | AB023582             |
| ccr-miR-430a| G protein-coupled receptor kinase               | Signal transduction       | AB119261             |
|             | NILT1 leukocyte receptor                        | Signal transduction       | AJ811994             |
|             | Retinol dehydrogenase 8                        | Metabolism                | AB439579             |
| ccr-miR-430b| Na+/glucose cotransporter                      | Metabolism                | JN867793             |
|             | Dsx and mab-3 related transcription factor 1–1 | Transcription factor      | KF713504             |
| ccr-miR-430c-3p| TNF receptor-associated factor 6 b            | Transcription factor      | HM535645             |
|             | Insulin-like growth factor binding protein 2    | Development               | FJ009601             |
| ccr-miR-365 | Rhesus blood group-associated glycoprotein C    | Development               | KF051940             |
| ccr-miR-2783| Cytochrome P450 aromatase                      | Metabolism                | EU499382             |
|             | Matrix metalloproteinase 2                      | Metabolism                | KC14857              |
| ccr-miR-3198| Mx3 protein                                    | Signal transduction       | AB027712             |
| cau-miR-3198| Progesterone receptor 1                         | Metabolism                | JX965185             |
| cau-miR-1814b| Nucleotide-binding oligomerization domain-2     | Signal transduction       | EY174419             |
| cau-miR-2742| Prominin-like protein                           | Metabolism                | DQ233501             |
| cau-miR-149 | Transmembrane protein 173                      | Metabolism                | JF970229             |
|             | Transcription factor 7-like 1a                  | Transcription factor      | FJ231713             |
| man-miR-4037| Sodium glucose cotransporter 1                 | Metabolism                | DQ285635             |
| man-miR-6751-3p| Vitellogenin 1                                | Development               | KF733650             |
| man-miR-7847-3p| Elongation factor 1- alpha                    | Transcription factor      | KF733649             |
|             | Doublesex and mab-3 related protein             | Transcription factor      | AB531495             |
|             | Glutamate dehydrogenase                        | Metabolism                | JF944444             |
| man-miR-142-3p| Vitellogenin 6                                | Development               | KF733655             |
|             | Elongation factor 1-alpha                      | Transcription factor      | KF733649             |
| man-miR-2452| Transferrin                                    | Metabolism                | JX292093             |
| man-miR-1603| HMG box transcription factor Sox8b             | Transcription factor      | GU166140             |
|             | Forkhead box L2                                | Transcription factor      | AB531497             |
| man-miR-2487| Sodium/potassium ATPase                       | Metabolism                | FJ982782             |
|             | Estrogen receptor alpha                        | Signal transduction       | EF530590             |
| mam-miR-10a-5p| MHC class I alpha chain                      | Development               | JF921124             |
| mam-miR-10a-3p| Spermatogenesis-associated protein 4           | Development               | JQ098682             |
| mam-miR-2369| Isolate LZ06 peroxisome proliferator          | Transcription factor      | HM140628             |
|             | Selenium-dependent glutathione peroxidase      | Metabolism                | KF378714             |
|             | Cardiac muscle troponin T isoform 2            | Metabolism                | KC556827             |
|             | Toll-like receptor 3                           | Signal transduction       | DQ986365             |
| cal-miR-4483| Lipoprotein lipase                            | Metabolism                | KC166231             |
| cal-miR-6852| Myosin heavy chain                            | Metabolism                | JX402919             |
| cal-miR-5600-3p| Myogenic differentiation antigen MyoD        | Transcription factor      | KC782835             |
Therefore, identification of the miRNA targets is an important step in understanding the miRNA regulatory function and gene regulation networks in five cyprinid fishes. The predicted targets for the identified miRNAs are shown in Table 2. A total of 46 target genes are predicted, of which 15 are from *C. carpio* miRNAs, 9 are from *C. auratus* miRNAs, 13 are from *M. anguillacea* miRNAs, 6 are from *M. amblycephala* miRNAs, and the rest 3 are from *C. alburnus* miRNAs. Our prediction of target genes for the five fish miRNAs discovered that more than one gene was regulated by individual miRNA, but only one gene targeted by miRNA was predicted individually in *C. alburnus*. The reason is the limited information on the *C. alburnus* miRNA transcripts in NCBI gene bank. Many experimental and/or computational approaches have documented that most of the miRNAs largely target transcription factors, signal transduction factors and development (Bartel, 2009; Friedman et al., 2009; Shibata et al., 2011). This study resulted in majority of the targets being classified as transcription factors, signal transduction factors and development (Bartel, 2009; Friedman et al., 2009; Shibata et al., 2011).

In this study, we applied this strategy to identify 23 miRNAs in five cyprinid fishes by searching both ESTs and GSS databases. Five random predicted miRNAs were validated by RT-PCR. These fish miRNAs potentially target 46 mRNAs, which can act as transcription factors, metabolism, development, and signal transduction. These findings will be helpful to elucidate their functions and processing of miRNAs from these fishes. The predicted miRNA targets reported in the present study are also required for validation in future studies. We believe that more miRNAs will be discovered from cyprinid fishes in future, with updated knowledge about miRNAs from fish species and availability of more complete fish genome sequences.

4. Conclusions

The computational approaches for identifying miRNAs and their targets play an important role in understanding gene regulation. In this study, we applied this strategy to identify 23 miRNAs in five cyprinid fishes by searching both ESTs and GSS databases. Five random predicted miRNAs were validated by RT-PCR. These fish miRNAs potentially target 46 mRNAs, which can act as transcription factors, metabolism, development, and signal transduction. These findings will be helpful to elucidate their functions and processing of miRNAs from these fishes. The predicted miRNA targets reported in the present study are also required for validation in future studies. We believe that more miRNAs will be discovered from cyprinid fishes in future, with updated knowledge about miRNAs from fish species and availability of more complete fish genome sequences.

Acknowledgments

This research was supported by Natural Science Foundation of China (31302013) and Doctoral Science Foundation (09001578) and Natural Science Innovation and Development Foundation (2013ZCX014) of the Henan University of Science and Technology.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sjbs.2015.05.007.

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