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In silico Discovery of Conserved and Novel miRNAs from Expressed Sequence Tags in the Chicken (*Gallus gallus*)

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ABSTRACT

MicroRNAs (miRNAs) and other types of small non-coding RNAs play a crucial role in the regulation of gene expression in eukaryotes either by post-transcriptional degradation or attenuating translation of messenger RNAs. In the case of the chicken (*Gallus gallus*), knowledge regarding miRNAs is still limited. In the present study, a computational approach was employed to screen miRNAs from the Expressed Sequence Tags (ESTs) of the chicken. A total of 21,298 known miRNAs belonging to 114 metazoan species were searched for homology against more than 192,020 EST sequences of the chicken. Consequently, 60 potential miRNA candidates were identified according to a range of filtering criteria. As a result, four novel miRNAs were found among the identified miRNAs including gga-miR-92a, gga-miR-2438, gga-miR-2970-5p, and gga-miR-2970-3p belonging to miR-92, miR-2438 and miR-2970 families. To predict their targeted genes, a BLAST search was done against the chicken 3' UTR mRNA database. As a result, 678, 422, 171 and 110 targets were determined for gga-miR-92a, gga-miR-2438, gga-miR-2970-5p, and gga-miR-2970-3p, respectively. Most of the predicted target genes participate in multiple biological processes, including immune system, regulation of cAMP biosynthesis, regulation of cyclase activity and regulation of lyase activity. Finally, a phylogenetic analysis of gga-miR-2970 and gga-miR-92a sequences revealed a close relationship between the chicken and *Taeniopygia guttata*, while gga-miR-2438 shares maximum percentage sequence similarity with bta-miR-2438 in *Bos taurus*. The present study is the first attempt to screen microRNAs from ESTs originating from the chicken leading to the identification of novel miRNAs.

Keywords: Computational approach, Filtering criteria, Phylogenetic analysis, Screening miRNAs.

INTRODUCTION

MicroRNAs (miRNAs) are small non-coding RNAs that suppress translation of target genes through cleavage of transcripts or attenuation of protein synthesis (Bartel, 2004; Bentwich, 2005). They are a class of single stranded non-coding RNAs (18-22 nucleotides in length) that were reported to be located primarily within non-coding regions of genomes in animals, plants and fungi (Bartel and Bartel, 2003; Carrington and Ambros, 2003; Hunter and Poethig, 2003; Lee *et al.*, 2004).

Most of miRNAs are generated from independent transcriptional units. They are 22-nt noncoding RNAs that regulate gene expression by associating with the multiprotein RNA-Induced Silencing Complex (RISC) and guiding RISC to silence specific mRNA species by mRNA degradation or translational inhibition

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(Bartel, 2009). Like protein-coding genes, *miRNA* genes are transcribed by RNA polymerase II, generating longer transcripts called primary miRNAs (pri-miRNAs). This initial product bears a 5' cap, a 3' poly-adenosine tail and often an intron that folds into hairpin structure(s) (Etheridge *et al.*, 2011). In the nucleus, pri-miRNAs are first cleaved by an RNase III endonuclease, Drosha, with the aid of DGCR8 to release hairpin-shaped precursor miRNAs (pre-miRNAs) usually ~55-70 nt in length. Pre-miRNAs are bound by the nuclear export factor Exportin 5 through their 2-3 nucleotide 3' overhangs and moved from nucleus to cytoplasm. Further, they are processed by cytoplasmic Dicer enzymes generating functional mature miRNAs (Lee *et al.*, 2003).

After discovery of miRNA in *Caenorhabditis elegans* in the early 1990s (Lee *et al.*, 1993), these regulatory non-coding RNA were identified across other species ranging from single cell (algae) to humans. It is estimated that 0.5 to 1 percent of the predicted genes consist of miRNAs in worms, flies, and humans (Sadeghi *et al.*, 2013), suggesting many regulatory roles in various biological activities and functions such as neural development (Chang *et al.*, 2004; Johnston and Hobert, 2003), cell division (Hatfield *et al.*, 2005), insulin secretion (Poy *et al.*, 2004), fat metabolism (Xu *et al.*, 2003), and tumor suppressors (Lee and Dutta, 2007; Tavazoie *et al.*, 2008) and oncogenes (Calin *et al.*, 2005).

Both experimental and computational approaches have been used to identify miRNAs. Experimental approaches are able to identify conserved and non-conserved miRNAs, however, they fail to detect rare miRNAs efficiently. Computational strategies have come to the aid of experimental methods in discovery of novel miRNAs. Computational strategies predict miRNAs by searching databases and exploiting evidences for conservation of miRNAs across multiple genomes. Evolutionary conservation of miRNAs provides a useful tool to predict new miRNA homologs and orthologs (Bartel and Chen, 2004; Lewis *et al.*, 2005; Weber, 2005; Zhao *et al.*, 2007). *In silico* methods employ some sequence- and structural features of miRNAs such as secondary structure characteristics, phylogenetic conservation of both sequence and structure, and thermodynamic stability of hairpins for prediction of novel miRNAs (Sheng *et al.*, 2011). Expressed Sequence Tag (EST) database has been broadly used for comparative genomics and miRNA prediction. Analysis of ESTs confers some advantages over other methods for miRNA prediction. In the absence of genome sequence, ESTs can be accessible alternative database for miRNA detection. Moreover, EST analysis provides evidence for miRNA expression, since ESTs are generated from messenger RNAs (Zhang *et al.*, 2008).

The chicken is one of the most important poultries in livestock. Billions of chickens are raised annually for both their meat and eggs. Knowledge of genetic background is necessary for application in the chicken's breeding programs. Previous studies have identified miRNAs in different livestock species such as sheep, cattle, and pigs using computational approaches (Sheng *et al.*, 2011; Zhou and LIU, 2010). In the chicken, the first set of 25 miRNAs was determined in embryos and adult chickens by small RNA cloning and sequencing (Xu *et al.*, 2006). Recently, several more attempts have been made to identify miRNAs from different tissues of the chicken. For instance, 47 miRNAs have been identified from chicken adipose tissue and skeletal muscle, 123 miRNAs from embryonic chicken livers, 449 miRNAs from chicken embryo and 33 miRNAs from skeletal muscle (Glazov *et al.*, 2008; Hicks *et al.*, 2010; Wang *et al.*, 2012; Yao *et al.*, 2011). Up to now, a total of 994 miRNAs from the chicken have been recorded in miRBase database (Release 21, June 2014). However, to our knowledge, there has been no previous report specifically examining publicly available EST data of the chicken for *miRNA* genes. Regarding the considerable economic importance of the

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chicken, the present study aimed to use all previously known miRNAs from different metazoan species and search for novel chicken miRNA homologs in all the publicly available EST sequences of the chicken.

MATERIALS AND METHODS

ESTs Sequences and Reference miRNA

A total of 192,020 EST sequences of the chicken were obtained from TGI database (http://compbio.dfci.harvard.edu/tgi/). The ESTs were assembled using locally installed assembly program CAP3 (Huang and Madan, 1999) into contigs (11,087 sequences) and singletons (163,898 sequences) to reduce inherent redundancy and to build unigene sets. Known mature miRNA sequences from different species of metazoan (up to 21,298 miRNAs belonged to 114 species, except *Gallus gallus*) were downloaded from the miRNA database miRBase (Release 21). To avoid inadvertent overlap of miRNAs, redundant miRNA sequences were removed. Then, closely related miRNAs were clustered together using CD-HIT-EST (Li and Godzik, 2006) and only one mature sequence was picked from each cluster. Out of the 21,298 known miRNAs, only 12,263 unique (non-redundant) miRNAs were selected as reference miRNAs for further analysis.

Identification of Conserved miRNAs in Chicken Using BLASTN

ESTs (unigene sets) were used for the identification of miRNAs in the chicken. To do this, a BLASTN search was performed for non-redundant miRNAs set against the ESTs. The E value cut-off for BLAST was 0.01 and the word-match size between the query and database was kept at 7. Then, the ESTs with more than 18 matched nucleotides and no-more-than-two mismatched nucleotides were considered as candidate ESTs containing miRNA.

In the next step, candidate miRNA precursors with approximate length of 220 nt were extracted (100 nt upstream and 100 nt downstream to BLAST hits by using local programs in perl script) from the candidate ESTs flanking the putative mature miRNAs. The protein coding sequences within these candidates were discarded by BLASTX search against Non-Redundant (NR) protein database (http://www.ncbi.nlm.nih.gov/). The sequences that had no hits at E-value 1e-6 were defined as non-coding ESTs and kept for further analysis. Out of the 170 candidate miRNAs precursor, 33 candidates fell under the protein coding category. In addition, miRNA precursors were searched against Rfam database (version10.1) (http://rfam.sanger.ac.uk/) using BLASTN software to remove non-coding RNAs such as rRNA, tRNA, snRNA, and snoRNA with the E value of 1e-6. As a result, 131 out of 137 candidate miRNAs were found as conserved miRNAs.

Prediction of Secondary Structure of Candidate Precursor miRNAs

The highly homologous candidate miRNAs identified in previous step were assessed for their secondary structure features including hairpin stem loop structure by MFOLD software (version 3.6) (Zuker, 2003). Later on, the following criteria were used to select potential miRNAs:

1. The candidate miRNA precursor sequences folding into an appropriate stem-loop hairpin secondary structure;
2. The minimum length of the pre-miRNA to be 50 nt;
3. The mature miRNA sequence should be placed on one arm of the hairpin structure;
4. Predicted mature miRNAs with no more than four nucleotide substitutions as compared to the known, reference miRNAs and the miRNA*miRNA

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duplex should not have more than six nt mismatches;
5. The A+U content of the candidate precursor miRNAs sequences should range from 30 to 70%;
6. Predicted secondary structure should have negative Minimal Folding free Energy (MFE) and positive Minimal Folding free Energy Index [MFEI= (MFE×100/Length of precursor miRNA)/(G+C%)]. The MFE value for each candidate precursor miRNAs should be less than -20 kcal mol^{-1}. MFE and MFEI are required to distinguish miRNAs from other small RNAs based on secondary structures.

Prediction of miRNA Targets and Their Functional Annotation

Potential targets of the predicted miRNAs were determined by a BLAST search for miRNA sequences against 3' UTR mRNA sequences of the chicken using two commonly used programs: RNAhybrid and miRanda. 3′ UTR sequences of the chicken transcripts in whole genome were obtained from ensemble gene 75. RNAhybrid is similar to an RNA secondary-structure prediction algorithm (e.g. MFOLD program) but it determines the most favorable hybridization site between two sequences. miRanda uses dynamic programming to search optimal sequence complementarities between a set of mature miRNAs and a given mRNA (Baloch and Din, 2014; Yousef *et al.*, 2009). To reduce false-positive results, alignments between each miRNA and its putative mRNA target were evaluated using the following parameters based on complementarity between them:

1. MFE was set as lower than −20 kcal mol^{-1}.
2. No mismatch in the region complementary to nucleotides 2-8 in the 5' end of miRNA that became known as the seed region.
3. The size of max internal loop (-u) and bulge loop (-v) was 3.

To identify the potential function of the novel predicted miRNAs, all targets of the miRNAs were subjected to DAVID functional classification tool (Database for Annotation, Visualization, and Integrated Discovery). DAVID is a web-based accessible program that performs a comprehensive set of functional annotations such as ontologies, protein domains, or pathways (Da Wei Huang and Lempicki, 2008). MiRNA targets were functionally categorized based on gene ontology annotation to provide insight into function of miRNAs (Wang *et al.*, 2009).

Validation of Predicted miRNAs by a Phylogenetic Analysis

A phylogenetic analysis was accomplished to determine evolutionary relationships between novel predicted miRNAs and collected precursor miRNAs. Novel candidate miRNAs were searched against all the known metazoan pre-miRNAs in miRBase database using BLASTN by allowing a maximum mismatch of 3 and e-value of 0.001. The precursor sequences of the homologous miRNAs were identified in miRBase database. Then, these were aligned with the precursor sequences of the predicted miRNAs in the same family using ClustalW and analyzed by MEGA 6 software (Maximum likelihood method based on Kimura 2-parameter substitution model) to investigate their evolutionary relationships (Tamura *et al.*, 2011).

RESULTS

Prediction of Potential miRNAs in the Chicken ESTs

A total of 60 miRNAs were derived from the chicken ESTs (174,985 sequences) that fulfilled all the criteria described in the Materials and Methods section and shared homology with 12,263 non-redundant mature miRNA. As a result, 56 out of 60

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Table 1. Characterization of the potentially novel identified chicken miRNAs. The novel identified chicken miRNAs were characterized in terms of Loc, Location of miRNA; LP, length of precursor miRNA; LM, length of mature miRNA; NM, number of mismatches between predicted and homologous miRNAs; MFE, minimal folding free energy; MFEI, minimal folding free energy index.

Loc	Chicken miRNA	Mature miRNA	Homologous miRNA	LP	LM	G+C %	MFE	MFEI
1	gga-miR-2970-5p	GACAGUCAGCAGUUGGUCUG	tgu-miR-2970-5p	62	21	58.1	23.6	0.65
1	gga-miR-2970-3p	AGAUCACCUCUUGGCUGUGGGU	tgu-miR-2970-3p	62	21	58.1	23.6	0.65
9	gga-miR-2438	GGGGUGUGUGCUGGAGCCUG	bta-miR-2438	84	19	61.9	36.5	0.70
4	gga-miR-92a	GGGUGGGGAUUUGUUGCAUUACU	oan-miR-92a-1-5p	62	23	50	23.3	0.75

miRNAs were experimentally annotated chicken miRNAs from chicken ESTs, indicating that our pipeline was a reliable method for exploring novel candidate miRNAs. Four miRNAs were found to be novel miRNAs that had never been annotated in the miRBase database (Table 1). Furthermore, the novel predicted miRNAs (potential precursors of miRNAs) were mapped to the chicken genome by Blat software (University of California, Santa Cruz [UCSC] Genome Browser, http://genome.ucsc.edu) (Kent, 2002). As a result, all of them were mapped to the genome (Table 1). These novel miRNAs were named gga-miR-92a, gga-miR-2438, gga-miR-2970-5p, and gga-miR-2970-3p. The names were assigned to the novel predicted miRNAs based on the naming scheme provided by miRBase database. The mature sequences were tagged 'miR', and the precursor miRNAs were labeled as 'mir' with the prefix 'gag' for *Gallus gallus*.

The novel mature and potential precursor of miRNAs had variation in length ranging from 19 to 23 and 62 to 84 nt, respectively (Table 1), which is in the range of the animal miRNAs. The analysis of nucleotide content in mature miRNAs shows a majority of G (39.0%), followed by U (29.7%), C (18.5%) and A (12.8%). In agreement with previous findings, the sequences of the miRNA precursors had A+U content ranging from 30% to 70% (Sheng *et al*., 2011; Zhou and Liu, 2010). Also, the G+C content of these pre-miRNAs was between 50-61.9% as seen in pig (Zhou and Liu, 2010). Figure 1 indicates the positions of miRNAs on the hairpins. The gga-miR-92a and gga-miR-2970-5p are derived from 5'-arm of the hairpin precursor, whereas the other two miRNAs are located on the 3'-arm.

Mfold software was used to predict secondary structure of these pre-miRNAs, showing that the flanking sequences of the predicted miRNAs were able to form the typical hairpin precursor structure (Figure 1). Based on the Mfold results, the negative MFE of precursor miRNAs varied over a range of -23.3 to -36.5 kcal mol^{-1}, whereas the average MFEI was of 0.69±0.02. As pointed by previous studies (Loong and Mishra, 2007; Zhang *et al.*, 2006), miRNA precursor sequences have significantly higher negative MFE and higher positive MFEI than other types of RNA e.g. tRNAs (0.64), rRNAs (0.59) and mRNAs (0.62–0.66) (Bonnet *et al.*, 2004). The secondary structures with lower MFE value are more stable thermodynamically (Prabu and Mandal, 2010).

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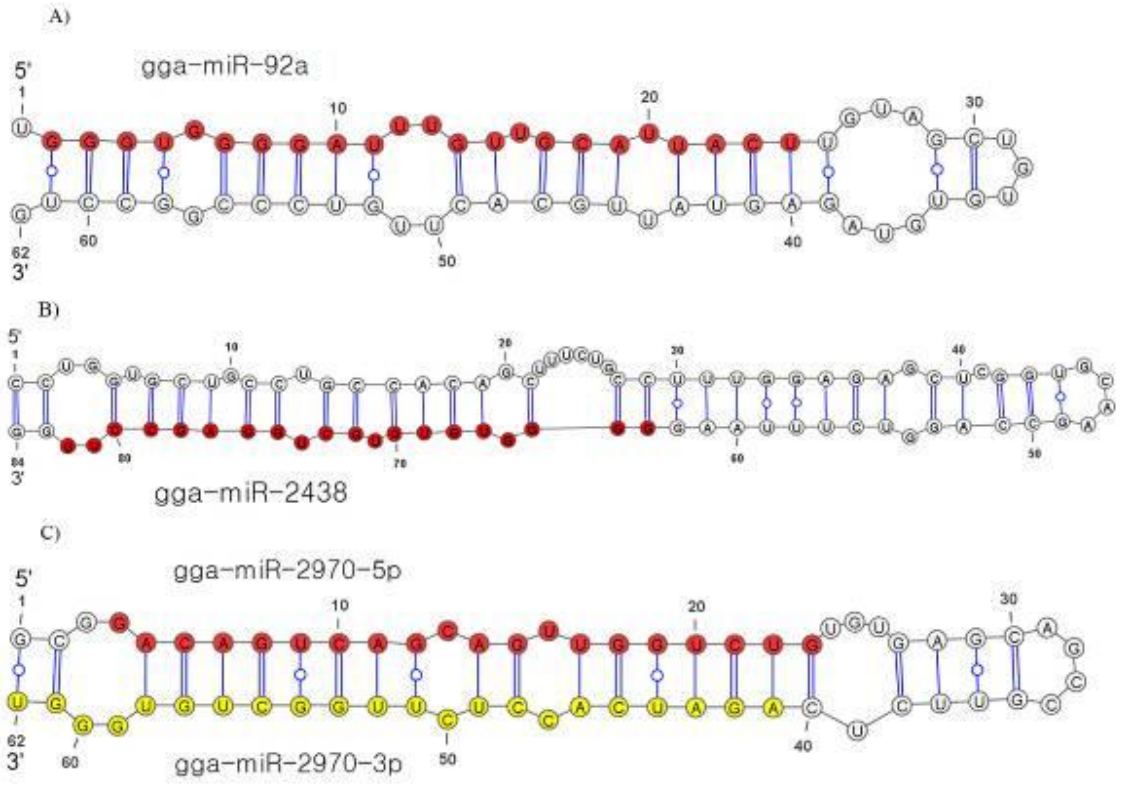

Figure 1. The predicted hairpin structures of potentially novel identified miRNAs along with mature and precursor sequences in the chicken: **(A)** gga-miR-92a (homolog of oan-miR-92a-1-5p); **(B)** gga-miR-2438 (homolog of bta-miR-2438), **(C)** gga-miR-2970-3p (homolog of tgu-miR-2970-3p) and gga-miR-2970-5p (homolog of tgu-miR-2970-5p).

Prediction of miRNA Targets

Here, two well-known software, RNAhybrid and miRanda, were employed for target prediction. Applying stringent criteria, targets predicted by both software were selected. A total of 678, 422, 171 and 110 potential targets were identified for gga-miR-92a, gga-miR-2438, gga-miR-2970-5p and gga-miR-2970-3p miRNAs, respectively, by searching against the 3' UTR mRNA sequences of the chicken (Supplementary 1).

GO Analysis

The miRNA-regulated genes are involved in a wide range of biological and metabolic processes (Pani and Mahapatra, 2013; Wang *et al.*, 2013). Here, to understand the miRNA-gene regulatory network and biological role of the predicted miRNA, their target genes were categorized based on their biological processes and molecular function. The results of GO (gene ontology) annotation are provided in Supplementary 2.

In biological processes category, target genes were found to be related with

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regulation of nucleotide biosynthetic process, regulation of cAMP biosynthetic process, positive regulation of growth, body morphogenesis and regulation of lyase activity. In molecular function category, most of the target genes were associated with carboxylic acid binding, peptidase activity, protein binding, calcium ion binding, and protein transporter activity.

Phylogeny of the Predicted miRNAs

A considerable amount of literature has demonstrated high conservation of the pre-miRNA and mature miRNA among distantly related species (Panda *et al.*, 2014; Sheng *et al.*, 2011; Zhou and Liu, 2010). The Phylogenetic tree was constructed to reveal evolutionary relationship between the identified novel precursor miRNAs in the chicken with their respective orthologs (Figure 2). The analysis revealed that gga-miR-2970 was similar to tgu-miR-2970 in the zebra finch and belonged to miR-2970 family. The 5′-arm and 3′-arm of the hairpin structure of gga-miR-2970 were identified on the same EST with 280 nucleotides in length (Figure 2). Also, gga-miR-92a was found to be a member of miR-92 family and is located on chromosome 4 in the chicken. Finally, gga-miR-2438 was placed on chromosome 9 of the chicken and belonged to miR-2438 family. There was no previous report of miR-2438 family in the chicken.

The phylogenetic analysis of gga-miR-2970 and gga-miR-92a sequences shows high similarity and evolutionary relationship with miRNAs reported in *Taeniopygia guttata* (Figure 2). The gga-miR-2438 shares maximum percentage sequence similarity with bta-miR-2438 (*Bos taurus)*.

DISCUSSION

Most of the mature miRNAs are evolutionary conserved in plant and animal species (Dezulian *et al.*, 2005; Weber, 2005). This conserved nature of the animal miRNAs has greatly enhanced the chance of finding conserved miRNAs using EST sequences in non-model organisms. In the present study, we aimed to search conserved miRNA in the chicken ESTs using a similar way. The applied strategy relies on two main features of miRNAs including conservation of mature miRNA sequence and secondary hairpin precursor structure of pre-miRNAs.

After discovery of miRNAs in metazoan species in 1993, (Lee *et al*. 1993; Wightman *et al*. 1993) a considerable number of studies have demonstrated their remarkable impacts on regulation of biological activities. Although high throughput tools such as next generation sequencing have identified a significant number of miRNAs in model and non-model species, accuracy of miRNA discovery is still challenging and requires interdisciplinary strategies such as computational methods. Huge amounts of biological data have led to development of mathematical algorithms for discovery of miRNAs computationally.

In the present study, we used a computational strategy to identify miRNAs by searching the EST database of the chicken and, following a set of strict filtering criteria, a total of 60 miRNAs were identified. The number of identified miRNAs is in good agreement with previous studies using the same approach in the sheep (Sheng *et al.*, 2011), cattle (Sadeghi *et al.*, 2013), pig (Zhou and Liu, 2010), earthworm (Gong *et al.*, 2010), *Catharnthus roseus* (Pani and Mahapatra, 2013) and garlic (Panda *et al.*, 2014). As a result, four potentially novel miRNAs were identified. Among novel predicted miRNAs, gga-miR-2438 and gga-miR-2970-5p have three and one nucleotides differences with the query miRNAs, respectively. These differences might be due to genetic mutation and possibly the long evolutionary distance between the chicken and other species (the zebra finch and cattle).

The miR-92 family has a large number of members (212 sequences in 81 species) compared to miR-2970 and miR-2438 families, according to miRBase database.

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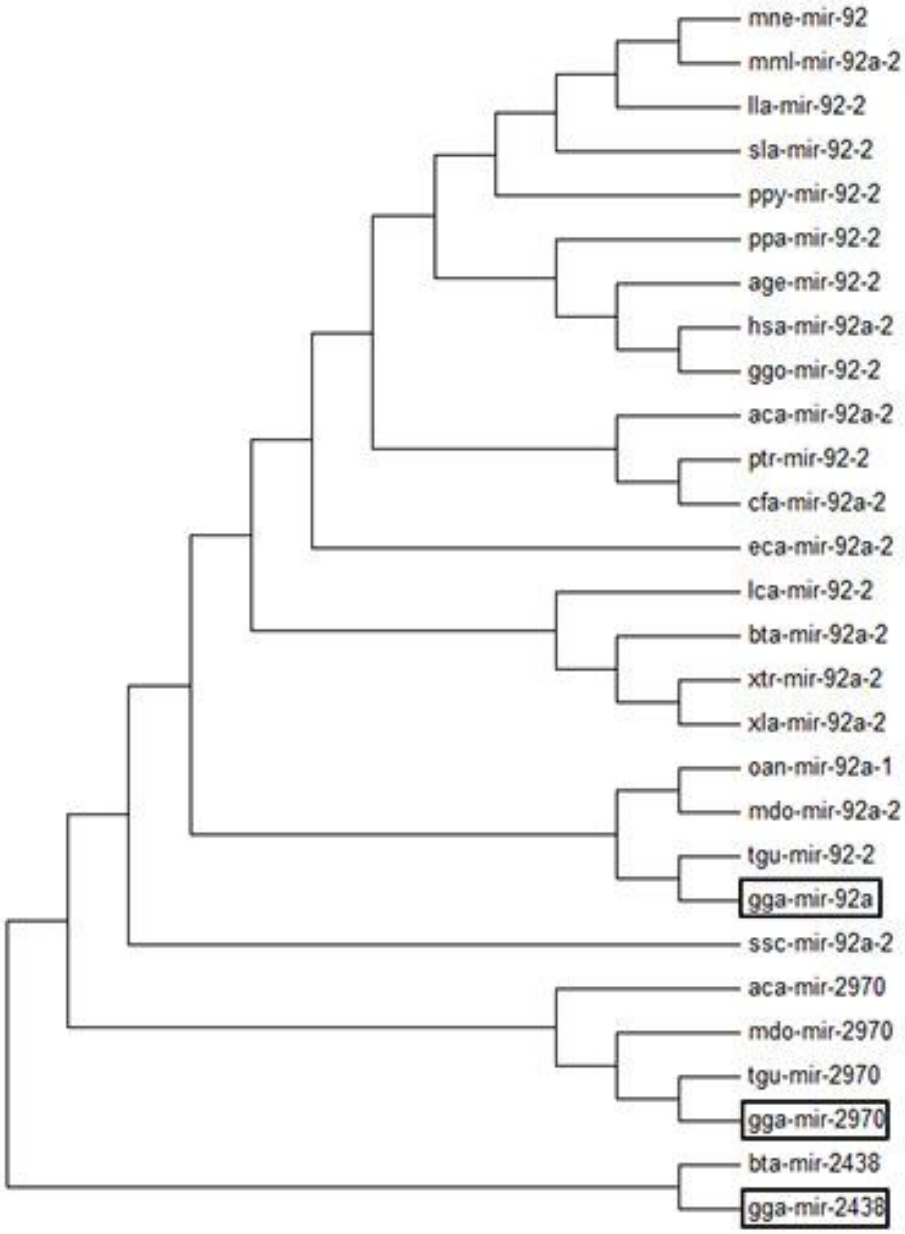

Figure 2. The chicken miRNAs phylogenetic analysis. The phylogenetic tree showed that chicken is more closed to zebra finch (*Taeniopygia guttata*) and cattle (*Bos Taurus*).

Based on our analysis, only four members of miR-2970 family were found in animal species including *Gallus gallus*, *Taeniopygia guttata*, *Anolis carolinensis*, and *Monodelphis domestica*. Additionally, just one member of miR-2438 family was only detected in *Bos taurus* (Figure 2).

In this study, phylogenetic tree was constructed to understand the relationship between the identified novel precursor miRNAs with that of other members of the same family. It is suggested that the chicken miRNAs have evolutionary relationships with miRNAs of different species. Furthermore, our results showed that different miRNAs evolve disparately not only within the same animal but also among different animals.

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miRNA target genes were predicted to understand the potential biological functions of miRNAs. In contrast to plant miRNAs, animal miRNA have limited complementary to their targets, conforming more complex miRNA/target secondary structures. Thus, we employed both RNAhybrid and miRanda programs to predict miRNA target genes accurately. miRNAs can regulate gene expression by binding to 5' or 3' UTRs of mRNA, however, most studies have demonstrated that animal miRNAs find their target mRNAs by binding to the 3' UTR (Vasudevan *et al.*, 2007; Wang *et al.*, 2013). Therefore, the 3' UTR of chicken mRNA sequences was used for target prediction.

Several predicted target genes of these novel miRNAs were found to be involved in immune system including BF2, MYD88, DCLRE1C and CCNB2 as gga-miR-2970-5p targets and THY1 as gga-miR-2970-3p target (Bradley *et al.*, 2009; Brandeis *et al.*, 1998; Qiu *et al.*, 2008; TÎrziu and Şereş, 2010). Interestingly, EST sequence containing gga-miR-2970 was detected in Bursa of Fabricius EST library. Bursa of Fabricius is a lymphoid organ that controls antibody-mediated immunity in the chicken and other birds (Cooper *et al.*, 1966).

CONCLUSIONS

Chickens are a global food source and an important model organism to study bird development. In our research work, for the first time, we employed an EST based homology search method with stringent criteria to identify novel miRNA in the chicken. Further research work can uncover functional roles of identified miRNAs during post-transcriptional events.

Supplementary 1. List of predicted target genes

Supplementary 2. Enriched GO terms of target genes of potentially novel identified miRNAs in the chicken: gga-miR-92a, gga-miR-2438, gga-miR-2970-5p and gga-miR-2970-3p.

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شناسایی میکرو آر ان ای های جدید و محافظت شده در توالی های برچسب دار و نشان دار شده مرغ (*Gallus gallus*)

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چکیده

میکرو آر ان ای ها و سایر آر ان های غیر کد کننده کوچک نقش مهمی در تنظیم بیان ژن ها چه در مرحله پس از رونویسی و چه در مرحله ترجمه آر ان ای پیامبر در یوکاریوت هابازی می کنند. در این مطالعه، ۱۹۲۰۲۰ از EST های جوجه برای یافتن میکرو آر ان ای ها تجزیه شدند. تعداد ۲۱۲۹۸ میکرو آر ان ای شناخته شده در این تجزیه شناسایی شدند که مربوط به ۱۱۴ گونه از جانوران هستند. تعداد ۶۰ کاندید میکرو آر ان ای احتمالی شناسایی شدند در نتیجه چهار میکرو آر ان ای جدید در بین این کاندید ها معرفی شدند که شامل: gga-miR-92a، gga-miR2438، gga-miR-2970-5p و gga-miR-2970-3p بودند. این میکرو آر ان ای های جدید متعلق به خانواده های miR-92، miR-2438و miR-2970 هستند. ژن های هدف این میکرو آر ان ای ها نیز پیش بینی گردید به طوری که ۶۷۸، ۴۲۲،۱۷۱ و ۱۱۰ ژن هدف به ترتیب برای gga-miR-92a، gga-miR2438، gga-miR-2970-5p و gga-miR-2970-3p پیدا شد. بسیاری از این ژن های هدف در فرآیندهای زیستی مختلف مانند سیستم ایمنی، تنظیم بیوسنتز cAMP، تنظیم فعالیت سیکلاز و لیاز نقش داشتند. تجزیه فیلوژنتیکی gga-miR-92a و gga-miR-2970 نشان داد که ارتباط نزدیکی میان جوجه و *Taeniopygia guttata* وجود دارد در حالی که gga-miR-2438 شباهت بسیار زیادی با bta-miR-2438 در *Bos Taurus* دارد. مطالعه حاضر برای اولین بار جهت جستجوی میکرو آر ان ای ها در داده های EST جوجه انجام شد.

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