Dhanoa et al. ExRNA (2019) 1:12
https://doi.org/10.1186/s41544-019-0013-1

ExRNA

RESEARCH Open Access

Discovery of isomiRs in PBMCs of diseased vis-à-vis healthy Indian water buffaloes

Jasdeep Kaur Dhanoa[1], Jasdeep Singh[1,2], Amarjit Singh[3], Jaspreet Singh Arora[1], R. S. Sethi[1] and C. S. Mukhopadhyay[1*]

Abstract

Background: MicroRNA isoforms are the variants of a canonical miRNA-sequence with alteration at the 3` or 5` termini due to addition, deletion and/or substitution of nucleotide. The present study aims at identifying the isomiRs of the expressed miRNAs in peripheral blood mononuclear cells (PBMCs) of diseased vis-à-vis healthy buffaloes, vis-à-vis exploring the molecular pathways of the predicted target genes of the isomiRs/miRNAs. Four groups of experimental animals (adult, male or female) were included in the study: a) Brucellosis (Murrah breed), b) Paratuberculosis or Johne's disease (Murrah breed); c) Brucellosis (Nili-Ravi breed) and d) control group of healthy buffaloes of the Murrah breed. The small RNA (sRNA) samples, extracted from PBMCs of each of the four groups, pooled into four samples and then were subjected to next-generation sequencing (Ion-Torrent PGM™ platform).

Results: The NGS data were analyzed using the miRanalyzer tool and R-programming to identify the differentially expressed (up and down-regulated (fold change ratio > 2)) miRNAs (exhibiting isomiRs and uniquely expressed miRNAs having isomiRs) of each of the three disease-groups as compared to the healthy-Murrah group. The target genes of these selected differentially and uniquely expressed miRNA & isomiRs were predicted using three different online tools (TargetScan, PicTar, and miRDB). These target genes were analyzed to determine their role in systems biology. We identified 153, 125 and 139 isomiR-exhibiting miRNAs that were common in those three experimental groups (healthy vs. Brucellosis-Murrah, Johne's disease-Murrah, and Brucellosis-Nili-Ravi, respectively). Gene ontology and pathway analyses of the target genes (Panther classification system) yielded several biological hits. Functional classification of the target genes indicated that these target genes were involved in different systems biology related functions like molecular binding, enzyme modulation, signal modulation, etc. The specificity of function was varying in the three experimental groups.

Conclusion: We are presenting the first report on the identification of isomiRs and functional classification of the target genes in water buffaloes. The results revealed that isomiRs may be involved in the biological processes and can be used in disease diagnosis.

Keywords: PBMCs, Next Generation Sequencing, miRNA, isomiRs, Gene Ontology

Background

MicroRNAs are single-stranded small noncoding RNA molecules of 22 bp, transcribed by RNA polymerase II [1, 2]. They play key role in post-transcriptional gene expression regulation [3] and thus, serve as negative regulator of numerous target genes [4]. Besides, miRNAs can affect gene expression through histone modification and DNA methylation of promoter sites. MiRNA silences the target mRNA molecules via base-pairing with complementary sequences at 3'UTR within the specific mRNA molecules [5]. It has been reported that miRNAs are associated with normal physiological and cellular processes and dysregulation in miRNA expression could result in liver, cardiovascular, kidney disorders or cancers [6, 7].

Plenty of reports are available on miRNA-repertoire in human, mice and some other lower animals (*Caenorhabditis elegans*, *Drosophila*). However, reports on the empirical identification of miRNAs in livestock are limited. Available

* Correspondence: csmbioinfo@gmail.com
[1]School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141004, India
Full list of author information is available at the end of the article

literature on RNAi-related works in the "black-gold" livestock-species Buffalo (*Bubalus bubalis*) is not available, except for the single research paper from our laboratory [8], where a total of 290 miRNAs were identified in bubaline-PBMCs out of which 130 were categorized as 'putative novel miRNAs'.

The isomiRs, i.e. the sequence variants of known miRNAs, are gaining importance in human, rat, mice and other species for their putative role in various diseases [9–11]. It is sometimes evident that the canonical sequence of miRNAs encoded by the same gene displays variation in length and sequence because of addition/deletion of one or more bases at any one or both the termini [12]. These are categorized as 5`-isomiRs (base change by addition or deletion at 5` end), 3`-isomiRs (base change by addition or deletion at 3` end) or mixed. In a recent study, it has been reported that uridylation and adenylation (i.e. addition of uridine and adenosine, respectively) to the 3′ ends of miRNAs play a major role in both stability and de-stability of RNA leading to change in its activity [13, 14]. It has been proven that isomiRs can influence miRNA stability by associating with target genes. The expression of miRNAs and isomiRs varies in male and female candidates [7]. Reports reveal the functional importance of isomiRs, for example, small RNA sequencing confirmed that 5`-isomiR-101 is responsible for decreased expression of some targets which indicated 5`-isomiR-101 as a functional variant in human tissues and cell lines [15]. Immunoprecipitation and luciferase assays showed that isomiRs get incorporated into Argonaute proteins, and their functions are different from their canonical miRNAs [12].

The present study aims to identify the isomiRs expressed in the PBMCs of the diseased (JD, Brucellosis) Indian water buffalo with respect to that of healthy ones. The putative target genes of the differentially expressed isomiRs have been predicted using online tools like PicTar, miRDB, and TargetScan. These target genes were further subjected to pathway analysis to explore their role in systems biology in animals.

Methods

Experimental samples and small RNA extraction

The isomiR-miRNA repertoire was compared in three disease groups: Brucellosis vs. Healthy in Murrah, Johne's disease vs. healthy in Murrah and Brucellosis infected Nili-Ravi vs. Healthy Murrah buffaloes. So, the experimental animals were divided into 4 groups a) Brucella infected buffaloes of Murrah breed (denoted as MuBr; $n = 2$), Johne's Disease infected buffaloes of Murrah breed (MuJD; $n = 5$), Brucella infected buffaloes of Nili-Ravi breed (NRBr; $n = 2$), and Healthy buffaloes of Murrah breed (MuHlthy; $n = 4$). The health status (diseased or healthy) was confirmed by Animal Disease Research Center, Department of Veterinary Pathology, GADVASU, Ludhiana. The PBMCs were isolated from 10 to 15 ml of fresh blood samples, using density gradient centrifugation and finally pooled for each of the four groups. The sRNA (enriched for miRNA) was isolated using *mir*Vana™ miRNA Isolation Kit (Ambion, Life Technologies, CA, USA). The four sRNA samples were sent to GCC Biotech Pvt. Ltd., Kolkata, for next-generation sequencing (NGS) followed by bioinformatics analysis of the raw sequencing data. The raw data was filtered based on the quality score (containing missing nucleotides and longer length). After filtering, all the produced reads were aligned to the reference genome assembly of the organism under study or to that of closest species (if genome assembly is not available for organism under study). The non-aligned reads were also removed from the further analysis part. The read sequences that were not aligned to mature miRNAs were obtained followed by mapping to the whole genome assembly for the discovery of novel miRNAs. Those cluster of reads were further analyzed following alignment pattern similar to the structure of miRNA precursor molecule (mature miRNA sequence – loop sequence – star sequence) along the reference genome, The genomic regions with such a pattern were extracted and run through Vienna package software to determine the RNA folding properties similar to hairpin structure. The procedure followed is mentioned in Additional file 1: Figure S1.

Analysis of miRNAs

Novel miRNAs were identified and validated by high throughput small-RNA (sRNA) sequencing using Ion Torrent PGM system (318 and 316 Chips). The sequencing data was used for analysis of 4 miRNA samples. In the current study, miRanalyzer software [16] was used for the analysis of miRNA and its variants. This tool determines the isomiR length and sequence variants by mapping the reads against known mature and pre-miRNA. It also maps the reads against other libraries of transcribed sequences, such as transcriptome, RFam, RepBase and eukaryotic tRNAs to discard messenger and other small non-coding RNAs [17]. The variants of these miRNAs (isomiRs) were identified using miRanalyzer. The stepwise workflow of the data analysis has been shown in Fig. 1:

I) **Expression profiling of miRNAs that exhibit IsomiRs:** The taurine miRNA sequences (premature and mature) were downloaded from the mirBase (www.mirbase.org) and then compared with the identified miRNAs (having isomiRs) in this study for each of the experimental groups using R-code (File: R Code isomiRs Compare by Groups.txt) [18]. miRDeep2 program and miRanalyzer tool were utilized for the identification of miRNAs and the

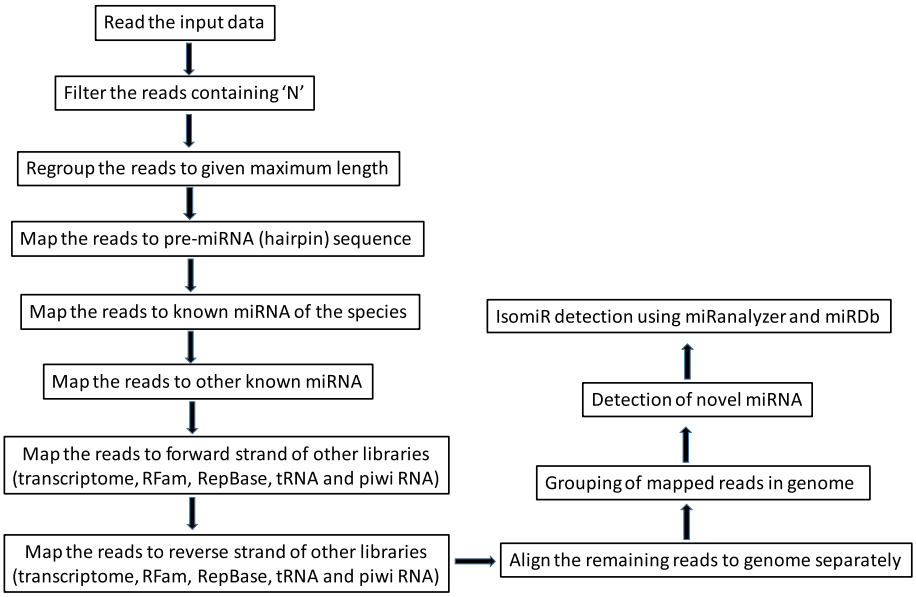

Fig. 1 Workflow used by miRanalyzer software

corresponding isomiRs from the sRNA sequencing data. The differentially expressed (i.e. up and down-regulated), as well as uniquely expressed miRNAs that exhibited IsomiRs, were detected for each of the three disease groups viz. Murrah breed suffering from Brucellosis (MuBr), Johne's disease positive Murrah buffaloes (MuJD) and Brucellosis-positive Nili-Ravi breed (NRBr) in comparison with the healthy Murrah buffaloes (MuHlthy) used as control group.

II) The Venn diagram was generated using R-packages gplots, gridGraphics and VennDiagram of R environment (File: R code JKDMS3 Venn Diagram 28,082,016.txt) to depict the common and uniquely expressed miRNAs in the experimental groups.

III) **Target prediction of top differentially expressed miRNAs that exhibit isomiRs**: The top 5 to 10 miRNAs (having isomiRs) displaying differential expression (ratio > 2) in the diseased sample (as compared to the healthy control buffaloes) and those showing unique expression in any one of the groups being compared were selected for predicting the putative target genes. Target prediction was done by online tools, namely, TargetScan (http://www.targetscan.org/vert_71/), PicTar (http://pictar.mdc-berlin.de/cgi-bin/PicTar_vertebrate.cgi) and MirDB (http://mirdb.org/miRDB/). The last two tools are based on available human-specific miRNAs.

IV) **Functional Annotation and Pathway analysis**: The putative targets predicted by those three tools were compared to identify the common target genes predicted by those three tools. The miRNA-target genes for each of the three experimental groups were subjected to gene ontology and pathway analyses using tools like Panther Classification System Ver. 11.1 (http://www.pantherdb.org/loginRequired.jsp?access=true) [19, 20] and Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (https://david.ncifcrf.gov/) [21, 22]. The role of the isomiR-exhibiting miRNA-target genes in systems biology are presented as pie-chart regarding their biological functions. The enrichment score of the graphs generated by DAVID to depict the gene functions is 1.0 with medium stringency. The gene-groups below 1.0 enrichment score have not been graphically depicted.

Results

1. **IsomiR identification:** The output of next-generation miRNA sequencing experiment also contains many sequences that are very similar with only a few nucleotides difference (additions/deletions). These sequences are termed as isomiRs i.e. presence of different forms of the same miRNA. In the study, a total of 274 miRNAs and 36 novel mature-star miRNAs were identified (Additional files 2 and 3). Out of these, the miRNAs that exhibited isomiRs have been determined from four different experimental groups (three disease groups and one healthy control). The common as well as unique miRNAs that exhibited isomiRs for each group were detected and diagrammatically represented by Venn Diagram (Fig. 2).

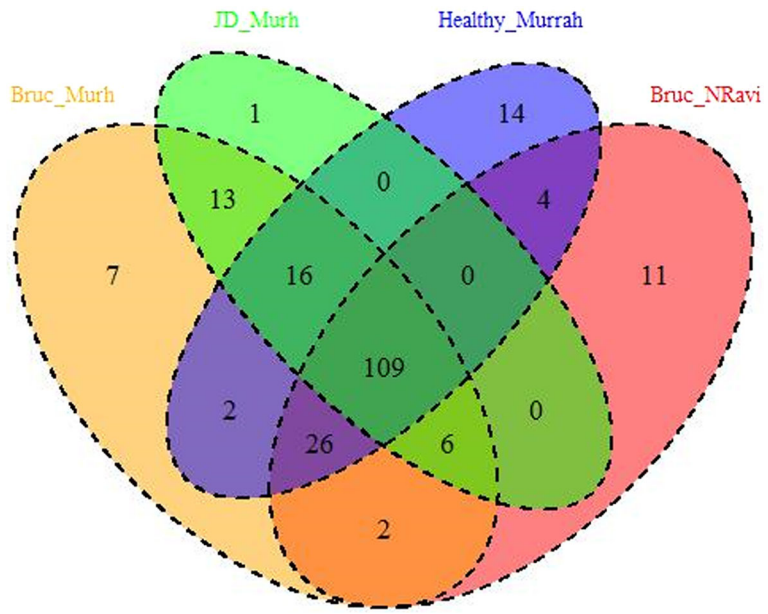

Fig. 2 Venn Diagram showing isomiRs of different groups

2. A total of 153 mature miRNAs (having isomiRs) were common in the first experimental group between the Brucella infected Murrah (MuBr) and the healthy control (MuHlthy). The log10 transformed expression was calculated for both mature and mature-star miRNAs exhibiting isomiRs for the diseased as well as healthy samples belonging to each of the three experimental pairs. The ratio of individual mature miRNAs of MuBr and MuHlthy was calculated to identify the up and down regulation of miRNAs. The ratio above 1 (> 1) and less than 1 (i.e. < 1) indicate up-regulated and down-regulated level of expression of miRNAs in disease-samples with respect to the healthy control samples (Additional file 4). In total, 28 unique miRNAs in the MuBr group (but not expressed in the control MuHlthy group) were detected (Additional file 5: Table S6). From these miRNAs three highly expressed miRNAs unique for MuBr group viz. bta-mir-29a, bta-mir-132, bta-mir-362 were also selected for further analysis (target prediction and functional analysis of target genes). Contrastingly, 18 unique miRNA present only in Healthy control animals (i.e. MuHlthy) were also identified (not present in the MuBr group) (Additional file 5: Table S7).
3. In the second experimental group, MuJD and the control MuHlthy were compared, and 125 common miRNAs that have isomiRs were detected (Additional file 6); out of which 20 miRNAs were detected unique to Johne's disease infected group only (not present in healthy control group) (Additional file 5: Table S8). While 46 uniquely expressed miRNAs were detected that were present only in the Healthy group not in JD infected group (Additional file 5: Table S9).
4. In the third experimental group, 139 miRNAs, that exhibit isomiRs, were common in Brucella infected buffaloes of Nili-Ravi (NRBr) and the healthy, control Murrah buffaloes (Additional file 7). Besides, 19 miRNAs were detected unique to NRBr samples (Additional file 5: Table S10) and 32 unique miRNAs were present only in Healthy buffaloes group (Additional file 5: Table S11).
5. **Gene Ontology:** Gene ontology is an in silico tool used for the representation of gene and gene products across all species. In the present study, miRNA and isomiRs were identified in different diseased and control groups. The predicted target genes have been shown in the Additional file 8 Predicted Targets for all the experimental groups. The top 2–3 differentially expressed and 1–2 uniquely expressed target genes have been selected for further functional classification. DAVID (Database for Annotation, Visualization, and Integrated Discovery) and Panther (The Protein ANalysis THrough Evolutionary Relationships) online tools were used for functional annotation and Gene Ontology categories (including molecular function and protein classification).

DAVID Results

DAVID enrichment of the predicted miRNA target genes has yielded two main gene-groups for the first experimental set (i.e. MuBr vs. Healthy control). Here, 14 and

nine genes were clustered in these two gene-groups, respectively, which demonstrate that most of the genes are associated with binding activities (nucleotide-binding, ATP-binding, ribonucleotide-binding, etc.) (Fig. 3). Besides these genes also exhibit kinase activities which are required for integrating biochemical signals and disease resistance [23].

DAVID analysis for functional classification of the target genes from the second experimental group (JD Murrah vs. Healthy Murrah) revealed that 4 and eight genes were clustered together for two gene-groups. These gene-groups are associated with primary functions including binding activities (DNA binding, nucleotide binding, ATP binding, ribonucleotide binding), helicase activities, kinase activity (tyrosine protein kinase, protein kinase,etc.) (Fig. 4). The kinase activity encoded by these genes are known to have defense response against various types of pathogenic infection [24].

In the third experimental group (Brucella NiliRavi (NRBr) vs. Healthy Murrah), DAVID analysis identified four different gene-groups with 4, 4, 19 and five genes clustered together, respectively. The genes from all the four groups are having transcription regulation and binding activities which are involved in pathological processes (Fig. 5). Instead of that, some genes are also responsible for apoptosis, programmed cell death, protein dimerization activities, and gene expression, etc. The steroid hormone receptor activity of gene has been found to be associated with various disorders [25].

Panther Classification of miRNA-target gene products

The gene ontology-based classification of the target genes also revealed that most of the proteins (corresponding to the target protein-coding genes) fall into the category of binding proteins having a role in calcium binding or cell adhesion etc. Besides the other important functional groups are having catalytic activity and receptor activity. These functions are necessary for growth and survival of cells [26].

The genes were categorized according to their molecular functions and protein classification. In the 1st experimental group Brucellosis Murrah vs. Healthy Murrah, the genes were sub-divided into seven different molecular functions and sub-classified into 21 different proteins (Fig. 6). For the 2nd experimental group, Brucellosis Nili-Ravi vs. Healthy Murrah, the genes were sub-divided into seven various molecular functions and sub-classified into 19 different proteins (Fig. 7). While in the 3rd experimental group, the JD Murrah vs. Healthy Murrah the genes were subdivided into eight different molecular functions and sub-classified into 22 different proteins (Fig. 8). From the molecular functions, binding and catalytic activity were covering the higher proportion (~ 70–75%) of the pie graph for all the three groups. In the third group, an additional antioxidant activity is present. On the other hand, the protein classification includes calcium-binding proteins, cell adhesion molecules, chaperones, hydrolase, ligase, signaling molecules, etc. For all the three groups nucleic acid binding was covering the higher proportion.

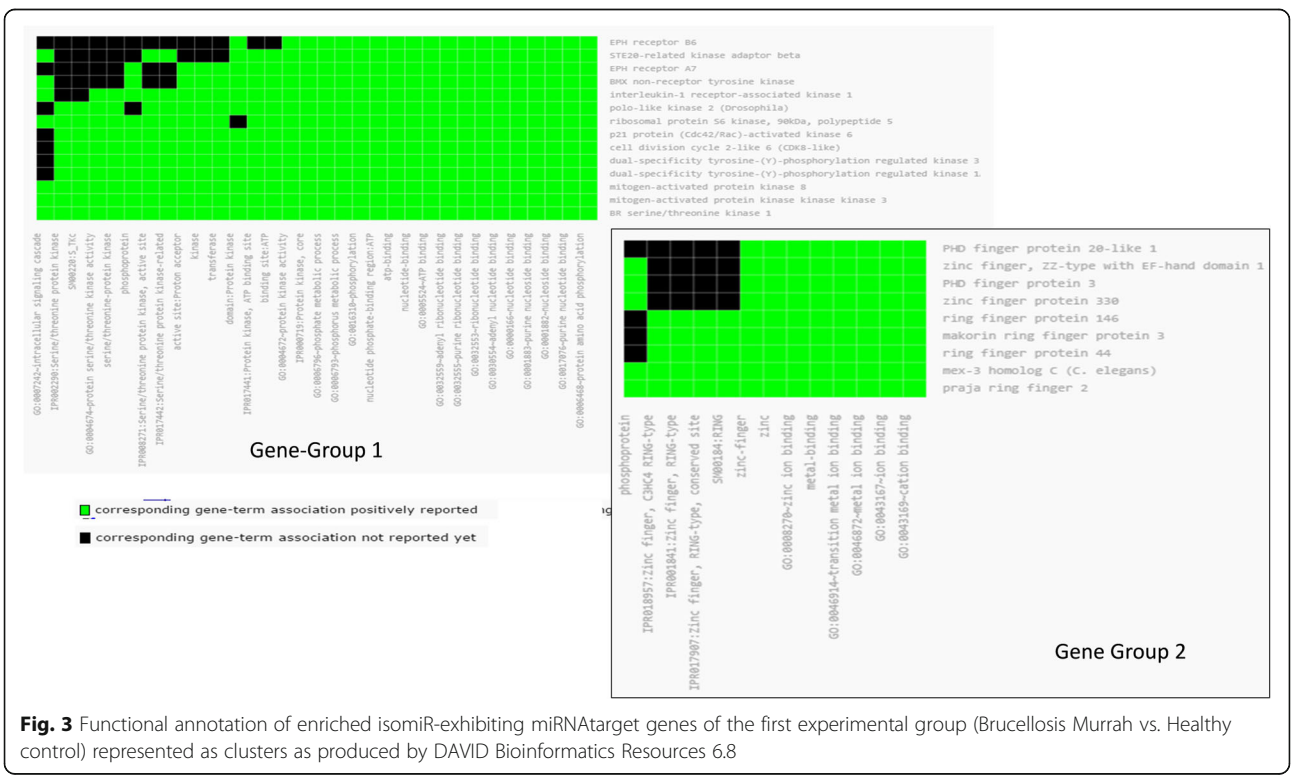

Fig. 3 Functional annotation of enriched isomiR-exhibiting miRNAtarget genes of the first experimental group (Brucellosis Murrah vs. Healthy control) represented as clusters as produced by DAVID Bioinformatics Resources 6.8

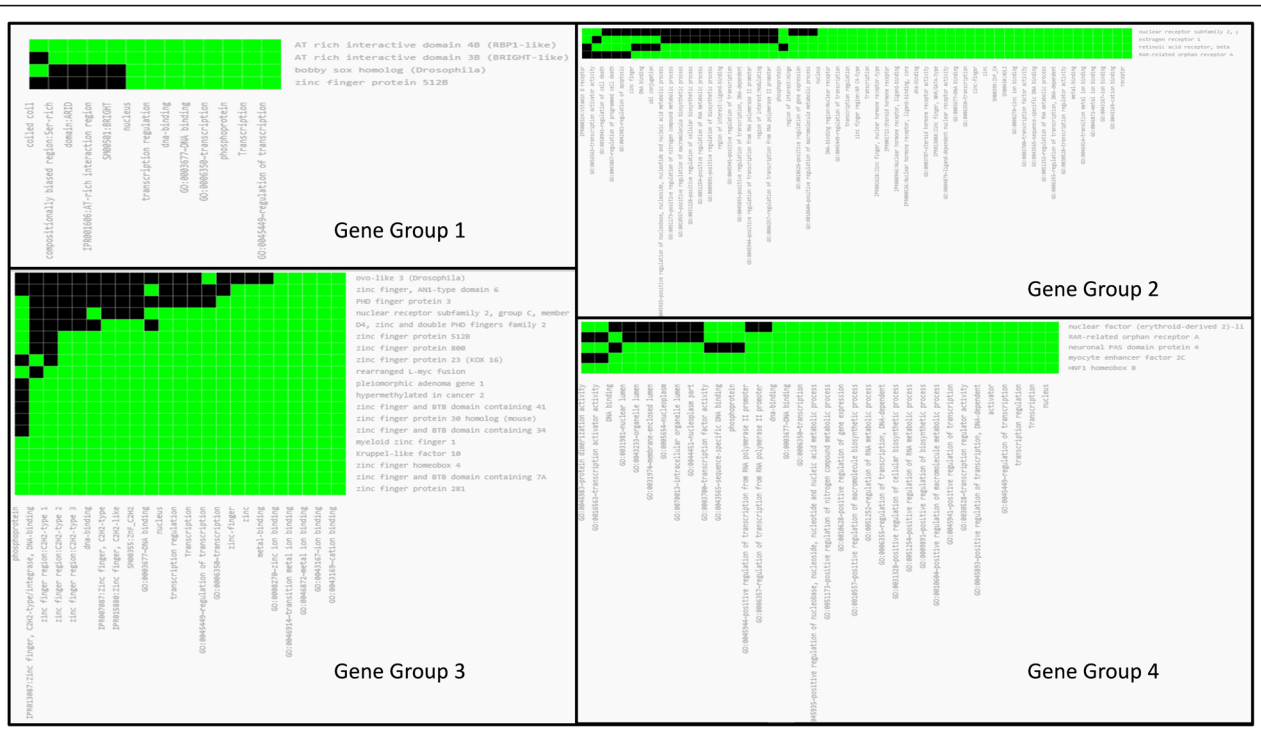

Fig. 4 Functional annotation of enriched isomiR-exhibiting miRNAtarget genes of the first experimental group (Johne's Disease Murrah vs. Healthy control) represented as clusters as produced by DAVID Bioinformatics Resources 6.8

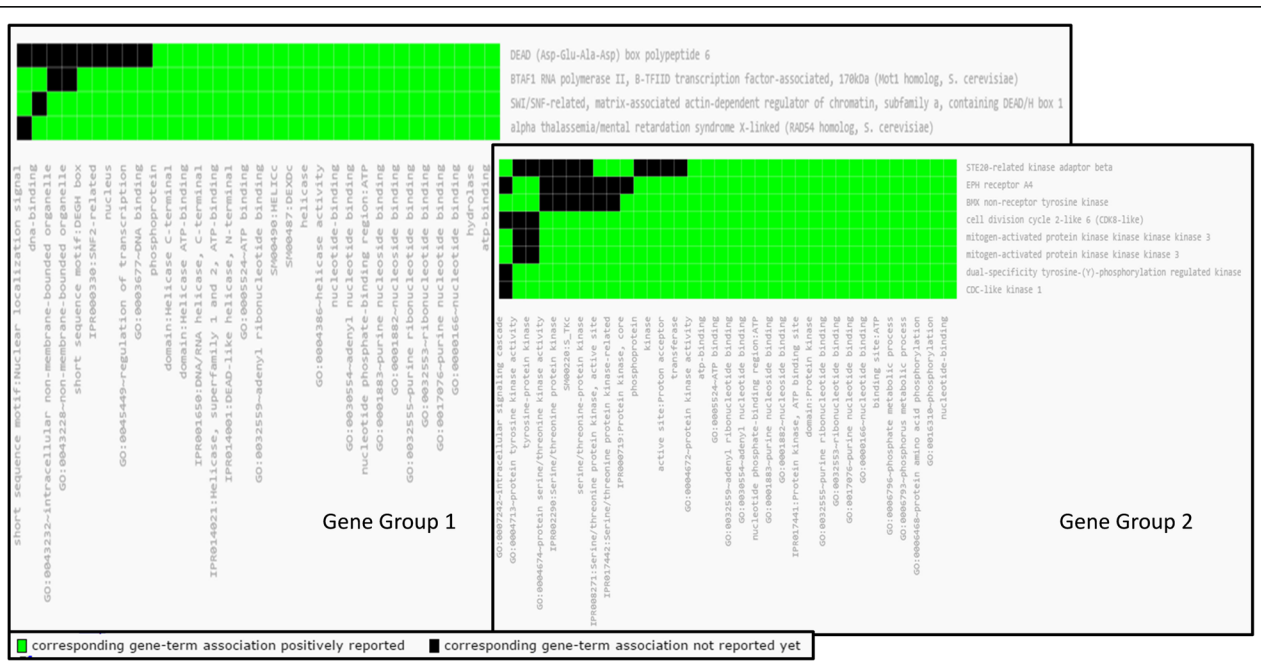

Fig. 5 Functional annotation of enriched isomiRtarget genes of the first experimental group (Brucellosis Nili-Ravi vs. Healthy control) represented as clusters as produced by DAVID Bioinformatics Resources 6.8

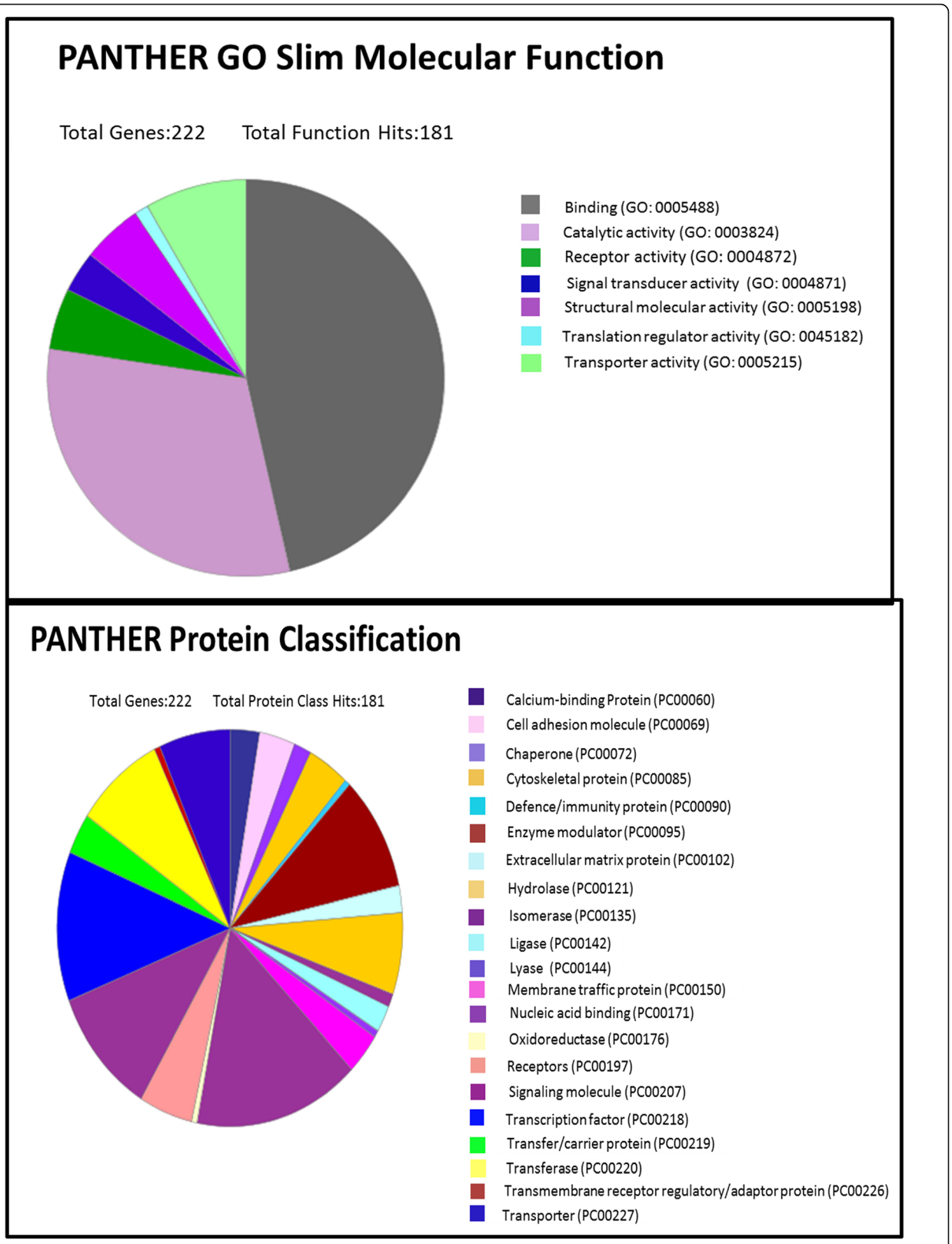

Fig. 6 Gene Ontology analysis and classification of the target geneson the basis of molecular function and protein classes for the experimental group 1 (i.e. Brucellosis-Murrah and Healthy control)

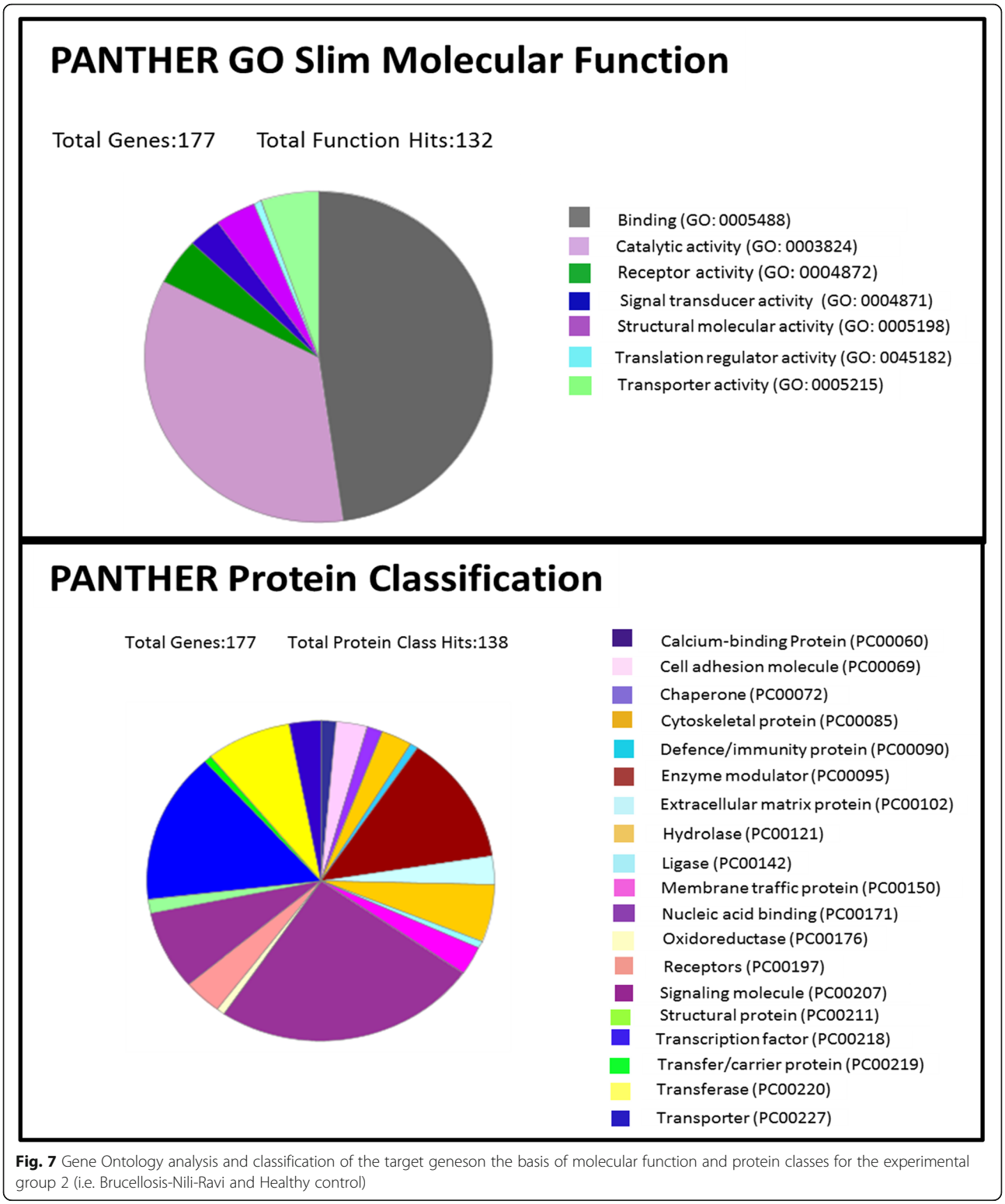

Fig. 7 Gene Ontology analysis and classification of the target geneson the basis of molecular function and protein classes for the experimental group 2 (i.e. Brucellosis-Nili-Ravi and Healthy control)

Discussion

Any change in canonical miRNA sequence (mature or mature-star) can affect the normal biological process of individual through change in expression level. This can have adverse effects including abnormal cell functioning and various types of disorders. The present era in the research filed is concentrating on the development and functioning of disease-specific target genes of these

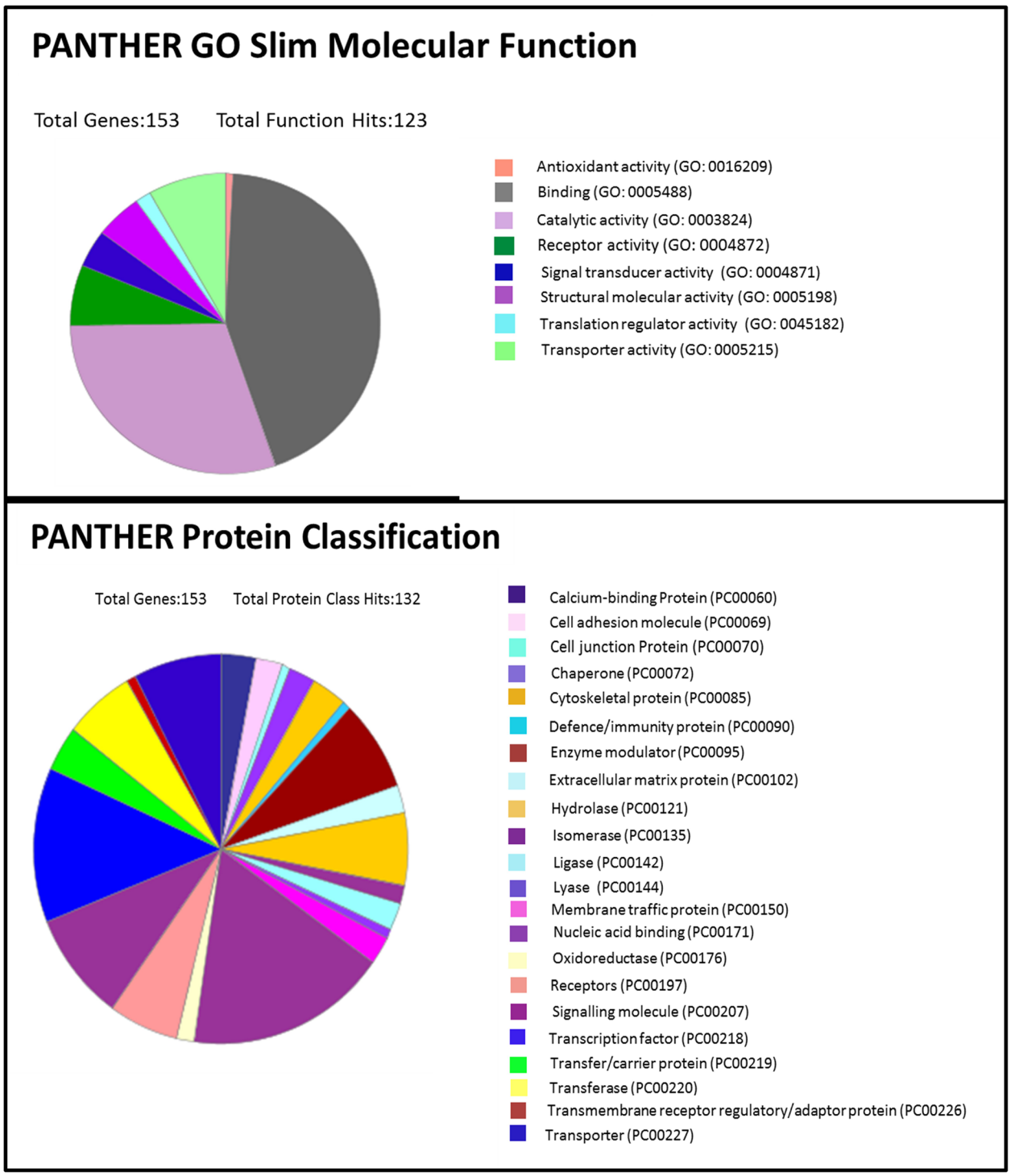

Fig. 8 Gene Ontology analysis and classification of the target geneson the basis of molecular function and protein classes for the experimental group 3 (i.e. Johne's Disease-Murrah and Healthy control)

miRNA which can be used for diagnosis purpose in future studies. If the variant of miRNA is present in the mature sequence, it will affect the targeting pathway of that specific miRNA. So, the investigation of the gene and pathway of that variant can provide new insight into disease diagnosis and pathogenesis. The miRNA study performed on whitefish (*Coregonus lavaretus*) revealed that exposure to microcystin-LR results in perturbation

on hepatic miRNA signaling pathway. The results concluded that miRNA and its variants can be used as markers for identification of liver-specific diseases in mammals [9]. Some miRNA and target genes, which are associated with human diseases are also well conserved in domestic animals. The report obtained from Gene Ontology results indicated that horse shares the highest homology to the human disease associated miRNA while chicken shares the least [27]. The expression level of the variants of these miRNAs (IsomiRs) can vary in response to biological stimuli [28].

The decreased levels of let-7, the first conserved miRNA detected in *C. elegans* has been detected to be involved in lung oncogenesis with increased RAS protein levels in normal lung tissue [29]. An ultra-deep sequencing study was performed using the miRNA-MATE pipeline for detection of canonical miRNA and their isomiRs in human tissues which indicated that both were sharing sequence and expression characteristics [30].

IsomiRs play major biological roles in disease diagnosis and pathogenesis. The study conducted on bacterial infection caused by *Mycobacterium* genus revealed the alteration in expression (including a change in relative arm expression and isomiR distribution across bacteria) of miRNA and their isomiRs. It is evident from our study that the underlying miRNAs that contribute to susceptibility/resistance of the host to the pathogenicity of mycobacterial infections can be identified from their internal variability in response to bacterial infection [31].

Adenylation of human miR-21, which is reportedly implicated in numerous human diseases, leads to its destabilization [32]. While adenylation of miR-122 has a stabilizing effect on this miRNA; demonstrated in the GLD-2 knockout mice in which non-canonical poly(A) polymerase responsible for the 3′ terminal monoadenylation of miR-122 is reduced selectively [33]. miR-122 is also found to be monoadenylated in human fibroblasts cells [34].

In the current study, miRNA and its variants that were common and unique against Healthy Murrah buffalo in different experimental groups (Murrah Brucellosis, Nili-Ravi Brucellosis, JD Murrah) were detected. In silico identification of mature miRNA and their validation through real-time PCR has also been reported from our laboratory [35]. The study revealed the biological role of target genes in different systems biology related functions including molecular binding, enzyme modulation, signal modulation, etc. In future, this type of study can be helpful in disease diagnosis and pathogenesis by comparing different disease groups with Healthy. Also, the mature and mature-star miRNA study can help detecting the most abundant change at 5` or 3` end or arm switching. Earlier, we have studied the expression of these miRNAs in TLR stimulated and non-stimulated PBMCs and recognized that some miRNAs were highly expressed while others were moderately expressed in both treatment and control groups [8]. This is the first report on experimental identification and validation of bubaline miRNAs.

The entropy-based method introduced by Wang and colleagues showed a significant change in the level of isomiRs in the early and late stage of Alzheimer disease [10]. miRNA and its variants can be used as diagnostic markers. In a recent study, a distinct bovine specific isomiR profile has been identified from stored bovine miRNA and study concluded that stored samples can be used later in disease analysis [36].

These days, various online software is available for the identification of isomiRs. Some of them are listed as-DeAnnIso, isomir-SEA, isomiRage, isomiRex, isomiRID, miR-isomiRExp, miRspring, SeqBuster and YM500. Every tool has some function. IsomiRID can identify 5` or 3` and polymorphic miRNAs from the canonical sequence, and also non-templated 5` or 3` end variations by mapping the sRNAs in the known pre-miRNAs. IsomiRs from different sequencing libraries can be compared using this tool [37]. IsomiRage can distinguish isomiRs using target-based prediction method [38]. DeAnnIso (Detection and Annotation of IsomiRs) can detect isomiRs from an uploaded sample and provides the presence of SNPs in miRNAs [39]. During the alignment of miRNA:mRNA, isomiR-SEA (isomiR seed Extension Aligner) encounters the mismatch positions in input tags of miRNA seed and distinguish different isomiRs [40]. miRPro, an online tool, is used for the identification of miRNA as well as isomiRs from human, mouse and chicken datasets. The features of this software include expression, quantification and read cataloging of miRNA with arm switching identification which is not available in various tools including omiRas and miRExpress [41].

Conclusions

This is the first comparative report on the differential expression profiling of bubaline isomiRs detected in PBMCs of diseased (Brucellosis and Johne's disease) animals, from two breeds of Indian water buffaloes. Analysis of the target genes of the differentially as well as uniquely expressed isomiRs in each of the three experimental groups indicates that the isomiRs have gotten a direct or indirect role on some critical biological processes.

Additional files

Additional file 1: Figure S1: Flow chart of procedure followed for IsomiR identification. (DOCX 49 kb)

Additional file 2: Table S1: IsomiR mature (Predominant). (DOCX 130 kb)

Additional file 3: Table S2: IsomiR mature star (Less expressed). (DOCX 20 kb)

Additional file 4: Table S3: Novel mature isomiRs common in Brucella Murrah and Healthy Murrah. (DOCX 45 kb)

Additional file 5: Table S6. Unique isomiRs present in Brucella Murrah but not in Healthy Murrah. **Table S7.** Unique isomiRs present in Healthy Murrah but not in Brucella Murrah. **Table S8.** Unique isomiRs present in JD Murrah but not in Healthy Murrah. **Table S9.** Unique isomiRs present in Healthy Murrah but not in JD Murrah. **Table S10.** Unique isomiRs present in Brucella Nili Ravi but not in Healthy Murrah. **Table S11.** Unique isomiRs present in Healthy Murrah but not in Brucella Nili Ravi. (DOCX 36 kb)

Additional file 6: Table S5: Novel mature Common isomiRs in JD Murrah and Healthy Murrah. (DOCX 50 kb)

Additional file 7: Table S4. Novel mature Common isomiRs Brucella Nili Ravi and Healthy Murrah. (DOCX 52 kb)

Additional file 8: Predicted targets of common and unique microRNAs of various groups. (XLSX 52 kb)

Abbreviations

DAVID: Database for Annotation, Visualization and Integrated Discovery; DeAnnIso: Detection and Annotation of IsomiRs; DNA: Deoxyribonucleic acid; isomiR-SEA: isomiR seed Extension Aligner; MicroRNAs: miRNAs; MuBr: Brucella infected buffaloes of Murrah breed; MuHlthy: Healthy buffaloes of Murrah breed; MuJD: John's Disease infected buffaloes of Murrah breed; NGS: Next Generation Sequencing; NRBr: Brucella infected buffaloes of Nili-Ravi breed; Panther: The Protein Analysis through Evolutionary Relationships; PBMCs: Peripheral blood mononuclear cells; RNA: Ribonucleic acid; sRNA: small RNA

Acknowledgments

Sincere thanks are extended due to Dr. R S Grewal (Incharge Dairy Farm, GADVASU) for immediate assistance in sample collection and to Dr. Joydeep Mitra (GCC Biotech, Kolkata, India) for providing bioinformatics support. The laboratory facilities provided by GADVASU, Ludhiana is sincerely acknowledged.

Funding

The financial support provided by the Science and Engineering Research Board (SERB)-Department of Science and Technology (DST), under the Ministry of Science of Technology, Government of India (SERC-LS-293-2011, File Number SR/FT/LS-22/2011) is sincerely acknowledged.

Availability of data and materials

The NGS data has been submitted to SRA of NCBI.

1. "miRNA-Seq data are available, without any restriction at NCBI-SRA: SRR3382688, SRR3382673, SRR3382604 and SRR3383406".
2. "All the data generated through miRNA-seq NGS has been used analyzed to identify the bubaline isomiRs in different experimental groups. The results have been represented in the tables of the manuscript. The expression values have been further analyzed to identify the differentially expressed genes. All the sequence files have been made available at NCBI-SRA, without any restriction".

Authors' contributions

JKD: Manuscript writing; JS: Sample collection, preparation, manuscript editing; AS: Disease diagnosis and sample identification; JSA: Manuscript editing; RS: Manuscript editing; CSM: Hypothesizing and Planning the work, Expression data Analysis. All authors read and approved the final manuscript.

Ethics approval

The permission from the Institutional Animal Ethics Committee (IAEC) was taken for collecting peripheral blood.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details

[1]School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141004, India. [2]Present address: Department of Experimental Medicine and Biotechnology, PGIMER, Chandigarh 160012, India. [3]Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.

Received: 31 October 2017 Accepted: 26 February 2019
Published online: 02 April 2019

References

1. Ambros V. The functions of animal microRNAs. Nature. 2004;431(2006):350–5. https://doi.org/10.1038/nature02871 PMID 15372042.
2. Zhou X, Ruan J, Wang G, Zhang W, Wang R. Characterization and identification of microRNA core promoters in four model species. PLoSComput Biol. 2007;3(3):e37.
3. Geisler A, Fechner H. MicroRNA-regulated viral vectors for gene therapy. World J Exp Med. 2016;6(2):37–54. https://doi.org/10.5493/wjem.v6.i2.37.
4. Yang G, Zhang L, Li R, Wang L. The role of microRNAs in gallbladder cancer. Mol Clin Oncol. 2016;5:7–13.
5. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem. 2010;79:351–79. https://doi.org/10.1146/annurev-biochem-060308-103103.
6. Lee LW, Zhang S, Etheridge A, Ma L, Martin D, Galas D, Wang K. Complexity of the microRNA repertoire revealed by next-generation sequencing. RNA. 2010;16(11):2170–80. https://doi.org/10.1261/rna.2225110.
7. Guo L, Liang T, Yu J, Zou Q. A Comprehensive Analysis of miRNA/isomer Expression with Gender Difference. PLoS One. 2016;11(5):e0154955. https://doi.org/10.1371/journal.pone.0154955.
8. Singh J, Mukhopadhyay CS, Kaur S, Malhotra P, Sethi RS, Choudhary RK. Identification of the MicroRNA Repertoire in TLR-Ligand Challenged Bubaline PBMCs as a Model of Bacterial and Viral Infection. PLoS One. 2016:e0156598. https://doi.org/10.1371/journal.pone.0156598.
9. Brzuzan P, Florczyk M, Łakomiak A, Woźny M. Illumina Sequencing Reveals Aberrant Expression of MicroRNAs and Their Variants in Whitefish (*Coregonus lavaretus*) Liver after Exposure to Microcystin-LR. PLoS One. 2016;11(7):e0158899. https://doi.org/10.1371/journal.pone.0158899.
10. Wang S, Xu Y, Li M, Tu J, Lu Z. Dysregulation of miRNA isoform level at 5′ end in Alzheimer's disease. Gene. 2016;584(2):167–72. https://doi.org/10.1016/j.gene.2016.02.020.
11. McGahon MK, Yarham JM, Daly A, Guduric-Fuchs J, Ferguson LJ, Simpson DA, Collins A. Distinctive profile of IsomiR expression and novel microRNAs in rat heart left ventricle. PLoS One. 2013;8(6):e65809. https://doi.org/10.1371/journal.pone.0065809.
12. Chin TG, Nicholas D. IsomiRs have functional importance. Malaysian J Pathol. 2015;37(2):73–81.
13. Song J, Song J, Mo B, Chen XM. Uridylation and adenylation of RNAs. Sci China Life Sci. 2015;58:1057. https://doi.org/10.1007/s11427-015-4954-9.
14. Xie F, Wang Q, Zhang B. Global microRNA modification in cotton (*Gossypium hirsutum L.*). Plant BiotechnolJ. 2015;13:492–500. https://doi.org/10.1111/pbi.12271.
15. Llorens F, Bañez-Coronel M, Pantano L, del Río JA, Ferrer I, Estivill X, Martí E. A highly expressed miR-101 isomiR is a functional silencing small RNA. BMC Genomics. 2013;14:104.
16. Hackenberg M, Sturm M, Langenberger D, Falcon-Perez JM, Aransay AM. miRanalyzer: a microRNA detection and analysis tool for next- generation sequencing experiments. Nucleic Acids Res. 2009;37:W68–76.
17. Singh J, Dhanoa JK, Choudhary RK, Singh A, Sethi RS, Mukhopadhyay CS. Discovery and comparative analysis of bubaline microRNAs in PBMCs of diseased vis-à-vis healthy Indian water buffalo. Computational Biology and Chemistry. In pipeline.
18. Friedländer MR, Mackowiak SD, Li N, Chen W, Rajewsky N. miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. Nucleic Acids Res. 2012;40(1):37–52. https://doi.org/10.1093/nar/gkr688.
19. Thomas PD, Campbell MJ, Kejariwal A, Mi H, Karlak B, Daverman R, Diemer K, Muruganujan A, Narechania A. PANTHER: a library of protein families and subfamilies indexed by function. Genome Res. 2003;13(9):2129–41.

20. Mi H, Guo N, Kejariwal A, Thomas PD. PANTHER version 6: protein sequence and function evolution data with expanded representation of biological pathways. Nucleic Acids Res. 2007;35(Database):D247–D52. https://doi.org/10.1093/nar/gkl869.
21. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009b;37(1):1–13.
22. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. Nature Protoc. 2009a; 4(1):44–57.
23. Myers MJ, Murtaugh MP. Cytokines in Animal Health and Disease. 1st ed: CRC Press; 1995. ISBN-10: 0824794354
24. Xue R, Wu J, Zhu Z, Wang L, Wang X, Wang S, et al. Differentially Expressed Genes in Resistant and Susceptible Common Bean (*Phaseolus vulgaris L.*) Genotypes in Response to Fusarium oxysporum f. sp. phaseoli. PLoS One. 2015;10(6):e0127698. https://doi.org/10.1371/journal.pone.0127698.
25. Honour JW. Diagnosis of Diseases of Steroid Hormone Production, Metabolism and Action. J Clin Res Ped Endo. 2009;1(5):209–26. https://doi.org/10.4274/jcrpe.v1i5.209.
26. Wieduwilt MJ, Moasser MM. The epidermal growth factor receptor family: Biology driving targeted therapeutics. Cell Mol Life Sci. 2008;65(10):1566–84. https://doi.org/10.1007/s00018-008-7440-8.
27. Buza T, Arick M, Wang H, Peterson DG. Computational prediction of disease microRNAs in domestic animals. BMC Research Notes. 2014;7:403.
28. Neilsen CT, Goodall GJ, Bracken CP. IsomiRs – the overlooked repertoire in the dynamic microRNAome. Trends Genet. 2012;28(11):544–9.
29. Niwa R, Slack FJ. The evolution of animal microRNA function. Curr Opin Genet Dev. 2007;17:145–50.
30. Cloonan N, Wani S, Xu Q, Gu J, Lea K, Heater S, Barbacioru C, Steptoe AL. MicroRNAs and their isomiRs function cooperatively to target common biological pathways. Genome Biol. 2011;12:R126.
31. Siddle KJ, Tailleux L, Deschamps M, Loh YHE, Deluen C, Gicquel B, et al. Bacterial Infection Drives the Expression Dynamics of microRNAs and their isomiRs. PLoS Genet. 2015;11(3):e1005064. https://doi.org/10.1371/journal.pgen.1005064.
32. Boele J, Persson H, Shin JW, Ishizu Y, Newie IS, Søkilde R, Hawkins SM, Coarfa C, Ikeda K, Takayama K-I. PAPD5-mediated 3′ adenylation and subsequent degradation of miR-21 is disrupted in proliferative disease. Proc Natl Acad Sci U S A. 2014;111:11467–72.
33. Katoh T, Sakaguchi Y, Miyauchi K, Suzuki T, Kashiwabara S-I, Baba T, Suzuki T. Selective stabilization of mammalian microRNAs by 3′ adenylation mediated by the cytoplasmic poly (A) polymerase GLD-2. Genes Dev. 2009;23:433–8.
34. Burns DM, D'Ambrogio A, Nottrott S, Richter JD. CPEB and two poly (A) polymerases control miR-122 stability and p53 mRNA translation. Nature. 2011;473:105–8.
35. Manku HK, Dhanoa JK, Kaur S, Arora JS, Mukhopadhyay CS. Biocomputational identification and validation of novel microRNAs predicted from bubaline whole genome shotgun sequences. Comput Biol Chem. 2017;70:96–106.
36. Shaughnessy RG, Farrell D, Riepema K, Bakker D, Gordon SV. Analysis of Biobanked Serum from a Mycobacterium avium subsp Johne's disease Bovine Infection Model Confirms the Remarkable Stability of Circulating miRNA Profiles and Defines a Bovine Serum miRNA Repertoire. PLoS One. 2015;10(12):e0145089. https://doi.org/10.1371/journal.pone.0145089.
37. de Oliveira LFV, Christoff AP, Margis R. isomiRID: a framework to identify microRNA isoforms. Bioinformatics. 2013;29(20):2521–3. https://doi.org/10.1093/bioinformatics/btt424.
38. Sablok G, Srivastva AK, Suprasanna P, Baev V, Ralph PJ. isomiRs: Increasing Evidences of isomiRs Complexity in Plant Stress Functional Biology. Front PlantSci. 2015;6:949. https://doi.org/10.3389/fpls.2015.00949.
39. Zhang Y, Zang Q, Zhang H, Ban R, Yang Y, Iqbal F, Li A, Shi Q. DeAnnIso: a tool for online detection and annotation of isomiRs from small RNA sequencing data. Nucleic Acids Res. 2016;44:W166–75. https://doi.org/10.1093/nar/gkw427.
40. Urgese G, Paciello G, Acquaviva A, Ficarra E. isomiR-SEA: an RNA-Seq analysis tool for miRNAs/isomiRs expression level profiling and miRNA-mRNA interaction sites evaluation. BMC Bioinformatics. 2016;17:148.
41. Shi J, Dong M, Li L, Liu L, Luz-Madrigal A, Tsonis PA, Del Rio-Tsonis K, Liang C. mirPRo – a novel standalone program for differential expression and variation analysis of miRNAs. Sci Rep. 2015;5:14617. https://doi.org/10.1038/srep14617.