An exploration of non-coding RNA in exosomes delivered by swine anterior pituitary

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Research article

Keywords: Anterior pituitary exosomes, MiRNA, LncRNA, CircRNA, Cross-talk

Posted Date: July 29th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-36112/v1>

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Abstract

Background: The anterior pituitary is a key endocrine organ both in animal and human being drawing much concern. Exosomes are extracellular secretory vesicles carrying proteins, lipids and small RNAs. Previous studies have demonstrated that they had regulatory function both physiologically and pathologically. However, information on exosomes from anterior pituitary remains unknown.

Results: In this study, we separated and identified exosomes from anterior pituitary of Duroc swine model for the first time. Total RNA was extracted and RNA-seq was performed, followed by a comprehensive analysis of miRNAs, lncRNAs and circRNAs. Resultantly, we obtained 343 known miRNAs and 73 novel miRNAs, 15545 lncRNAs and 494 circRNAs. Furthermore, GO and KEGG enrichment analysis showed that the ncRNAs in exosomes may participate in regulating intracellular signal transduction, cellular component organization or biogenesis, small molecule binding, transferase activity. The cross-talk between them also suggested that they may play an important role in signaling process and the biological regulation.

Conclusions: This work firstly provides ncRNAs data in anterior pituitary exosomes from duroc swine breed. These results may serve as a fundamental resource for exploring the detailed functions of exosomes from anterior pituitary.

Background

The pituitary gland is often regarded as the “master gland”, coordinating the complex functions of multiple endocrine glands along with the hypothalamus [1]. The anterior pituitary, the glandular, anterior lobe of pituitary, is a very important organ of the endocrine system that regulates several physiological processes including cell generation cycle, stress, growth, reproduction, bone metabolism and lactation[2-6]. We have all known that it makes up more than 80% of the pituitary gland and secretes six major hormones, including growth hormone (GH), prolactin (PRL), adrenocorticotropin hormone (ACTH), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), which are crucial to our physiological well being[7, 8]. Many researchers focused on the hormones secreted by anterior pituitary that act on target organs including the adrenal gland, liver, bone, thyroid gland, mammary glands, ovaries and testes, and itself is regulated by the hypothalamus and by negative feedback from these target organs [1, 2, 4].

Exosomes are a nano-scale vesicle structure that can be secreted by most eukaryotic cells and their diameter size are about 30 to 150 nanometers (nm)[9, 10]. Exosomes are usually cup-shaped or round phospholipid bilayers under transmission electron microscopy, and are mainly spherical in body fluids. Exosomes are present in various tissues and biological fluids including blood, dendritic cells, lymphocyte, epithelial cells, red blood cells, stem cells, hepatocyte and various tumor cells[11-19]. They carry a cargo of biological molecular of their cell of origin, including proteins, lipids, mRNA, microRNA (miRNA), long non-coding RNA (lncRNA) and circular RNA (circRNA) [20-23]. The latest data from the Exocarta database

show that 9769 proteins, 3408 mRNAs, and 2838 miRNAs have been identified in exosomes of different cellular origin (<http://www.Exocarta.org>). Although it was previously considered a waste protein produced by cell metabolism[24], researchers found that exosomes have immunoregulatory functions and can be used as an important cell regulatory factor in the 1990s [16]. More and more evidences indicate that exosomes have a variety of functions in intercellular communication, which can be involved in material transfer, signal transduction and regulation of immune response[25-28]. Recently, Zhang et al reported pituitary tumor exosomes inhibits the growth of pituitary adenoma by transmitting lncRNA H19[29].

Non-coding RNA (ncRNA) is a functional RNA molecule that is not translated into proteins. miRNA is a small non-coding RNA molecule and can negatively regulate the expression of its target gene expression at post-transcriptional level[30-32]. MiRNAs have been identified involved in the development of the pituitary gland and participated in the process of regulation[33-44]. lncRNA is a type of non-coding RNA, defined as being transcripts with longer than 200 nucleotides[45] and researches indicated that lncRNAs play an important part in various biological processes[46, 47]. Many lncRNAs have been proved to work in pituitary adenomas and normal anterior pituitary[48-51]. circRNA is a class of single-stranded RNA that forms a covalently closed continuous loop. They have been categorized as non-coding RNA, but more recently, they have been shown to code for proteins and could serve as miRNA sponges, sequestering miRNAs by competitive to combine with targeted mRNAs[52-54]. A lot of researches have characterized circular RNAs by sorting through vast collections of RNA sequencing data[53, 55-57]. Recently, Li et al identified 10226 circRNAs from pituitary gland of prenatal and postnatal sheep through RNA-seq [58]. And some other studies have reported about circRNAs in pituitary adenomas [59, 60].

As an important endocrine organ, information about exosomes secreted from anterior pituitary is still very limited. In this study, for the first time we extracted and identified the exosomes from anterior pituitary of Duroc swine breed and then we make preliminary exploration of non-coding RNAs of them. This study will lay a basis for further exploration of functions of pituitary exosomes.

Methods

Sample collection and exosomes isolation

This study used 3 health male swines (Duroc), at age of 60-day-old, which were purchased from Jintuan farm of JIADA GROUP (Zhaoqing, Guangdong, China). Incubate the pig with an endotracheal tube (30 cm length, 8 mm ID) and anesthetize pig with isoflurane (4.5% of tidal volume by mask)[61]. Then, the pigs were euthanized by exsanguination under a surgical plane of the isoflurane anesthesia[62].

We removed the pituitary glands and the anterior lobe was immediately dissected from each pituitary gland under sterile conditions. The anterior pituitary glands were washed with phosphate-buffered saline (PBS). We cut the tissue and then minced tissues were cultured with Dulbecco's Modified Eagle's Medium/Nutrient Mixture F12 (DMEM/F12) (Gibco, US) supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, US). Forty-eight hours later, media was harvested for exosome isolation. Culture media was mixed with ExoQuick precipitation solution, and exosomes isolation were performed

according to the manufacturer's instructions (SBI System Biosciences, CA, USA) as described previously [63-70] and then we use 0.22- μ m filter to obtain final sample. All animal experimentation complied with the

laboratory animal management and welfare regulations approved by Standing Committee of Guangdong People's Congress (Guangzhou), China.

Electron microscopic analysis of exosomes

A drop of exosome suspension (about 10 μ L) was fixed on formvar-coated copper grids for 2 min, washed briefly in ultrapure water, negatively stained with 1% uranylacetate and observed by transmission electron microscopy (TEM; JEM-2000EX; Jeol, Tokyo, Japan) at an acceleration voltage of 80 kV.

BCA Protein Assay, SDS-PAGE and Western Blot Analyses

We assayed total protein content using the Pierce BCA Protein Assay Kit (ThermoScientific, Waltham, MA) according to the manufacturer's instructions. The proteins were measured using a FluorChem M Fluorescent Imaging System (ProteinSimple, Santa Clara, CA), separated by SDS-PAGE (10%) and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA). We used two positive markers (CD9 and CD63) for Western blots. After blocking with 5% skim milk for 2 h, the membranes were incubated overnight at 4°C with specific antibodies against CD9 and CD63 (1:1,000; Sangon Biotech, China). We applied horseradish peroxidase-conjugated goat anti-rabbit IgG (H+L;1:50,000;Jackson ImmunoResearch, West Grove,PA) as a secondary antibody for 1 h at room temperature.

Total RNA extraction, RNA-Seq library preparation and sequencing

We extracted total RNA from exosome suspension samples using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. The RNA quantity and quality were assessed using an RNA 6000 Nano Lab-Chip Kit and Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA) with an RNA integrity number > 7.0. A total amount of 3 μ g total RNA per sample was used as input material for the small RNA library. Sequencing libraries were generated using NEBNext® Multiplex Small RNA Library Prep Set for Illumina® (NEB, USA). After cluster generation, the library preparations were sequenced on an Illumina HiSeq 2500/2000 platform and 50bp single-end reads were generated at the Novogene Bioinformatics Institute (Beijing, China). A total amount of 20 ng RNA per sample was used as input material for the Library preparation for lncRNA sequencing. The libraries were sequenced on an Illumina HiSeq 2500 platform and 125 bp paired-end reads were generated. A total amount of 5 μ g RNA per sample was used as input material for the library preparation for circRNA sequencing. The libraries were sequenced on an Illumina HiSeq 4000 platform and 150 bp paired-end reads were generated.

Sequence Data analysis

For small RNA sequencing, raw reads of fastq format were filtered through custom perl and python scripts at first. Clean reads were obtained by removing reads containing ploy-N, with 5' adapter

contaminants, without 3' adapter or the insert tag, containing poly A or T or G or C and low quality reads from raw data. Q20, Q30, and GC content of the clean data were calculated at the same time. High-quality data were used to do the subsequent analyses. The small RNA tags were mapped to reference sequence by Bowtie [71] without mismatch to analyze their expression and distribution on the reference. Mapped small RNA tags were used to looking for known miRNA. miRBase20.0 was used as reference, modified software mirdeep2 [72] and srna-tools-cli were used to obtain the potential miRNA and draw the secondary structures. The characteristics of hairpin structure of miRNA precursor can be used to

predict novel miRNA. The available software miREvo [73] and mirdeep2 [72] were integrated to predict novel miRNA through exploring the secondary structure, the Dicer cleavage site and the minimum free energy of the small RNA tags unannotated in the former steps.

For lncRNA sequencing, raw reads of fastq format were firstly processed through in-house perl scripts. Then we obtained clean reads by removing low-quality reads and those containing adapters and poly-N from the raw data. At the same, Q20, Q30, and GC content of the clean data were calculated. Index of the reference genome was built using bowtie2 v2.2.8 and paired-end clean reads were aligned to the reference genome using HISAT2 v2.0.4 [74]. The mapped reads of each sample were assembled by StringTie (v1.3.3) in a reference-based approach [75]. So after evaluating the quality of original data produced, we set up a series of strict screening conditions according to its structural and functional characteristics based on the results of transcriptome splicing. Through the following 5 steps of screening: exon number screening, transcript length screening, known transcript annotations screening, transcript expression screening and coding potential screening, the screened lncRNA was taken as the final candidate lncRNA set for subsequent analysis. Then we use three types of coding potential analysis software CNCI [76], CPC2 [77] and Pfam-scan [78] to distinguish lncRNA from mRNA. The intersecting results of each software were defined, and those that were determined to be noncoding were designated as candidate lncRNA. We used Cufflink (v2.1.1) to calculate fragments per kilobase million (FPKM) for both lncRNA and coding genes [79]. The transcript expression levels (FPKM value) were expressed as fragments per kilobase of transcript per million mapped reads values.

For circRNA sequencing, the procedure of quality control and mapping to the reference genome is same with lncRNA sequencing. The circRNA were detected and identified using find_circ [80] and CIRI2 [81]. Circos software was used to construct the circos figure and the raw counts were first normalized using TPM [82].

We used KOBAS [83] software to test the statistical enrichment of the target gene candidates in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. On the other hand, we did ceRNA analysis, screened miRNAs and selected mRNAs, lncRNAs and circRNAs that have a targeted relationship with the miRNA and have negative correlations in expression using miRanda. Cytoscape software was used to construct the lncRNA-miRNA-gene and circRNA-miRNA-gene networks.

Results

Isolation and identification of exosomes from anterior pituitary of Duroc swine

Exosomes were isolated from Duroc swine anterior pituitary (Additional Fig.1). We detected the purified vesicles using transmission electron microscopy which showed that their size and morphology in cup-shaped (Fig.1a), typical of that of exosomes. Then we used the Zetasizer to analyze their size distribution and found that the vesicles average size is about 92nm (Fig.1b). Moreover, exosomes were confirmed by Western Blot with positive common surface markers CD9 and CD63 (Fig.1c).

Overview of small RNA deep sequencing data in exosomes and analysis

In order to explore the non-coding RNA expression profiles of the exosomes from anterior pituitary of Duroc swine breed, we used RNA-seq analyses to characterize the non-coding RNA from three normal anteriors of 60-day-old Duroc swine. We obtained 12778982 (Exo_1), 15668033 (Exo_2) and 1535301139 (Exo_3) clean reads that were screened from sRNA for subsequent analysis after quality evaluation (Additional file 1: Table S1). Meanwhile, the length distribution of the obtained total sRNA fragment was statistically analyzed (Additional Fig.2). In general, sRNAs range from 18 to 35nt in length and the majority of the miRNA reads were about 22 nt. A total of 416 miRNAs were obtained from samples, 343 of which are known miRNAs and 73 are newly predicted miRNAs (Additional file 2: Table S2). Of these known miRNAs, 61 miRNAs were highly expressed ($1,000 < \text{average signals} \leq 10,000$), and, in particular, 46 miRNAs were extremely highly expressed in exosomes (average signals $\geq 10,000$). To further characterize the regulatory roles of miRNAs in exosomes from anterior pituitary, miRNA target prediction, Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation analyses were performed in our study. A total of 185183 target genes for the 416 miRNAs were predicted. Our GO annotation indicated that the predicted target genes were significantly enriched in intracellular signal transduction, phosphorylation, catabolic process, developmental process, the component of cytoskeletal part, binding, protein binding and nucleotide binding (Fig.2a and Additional file 3: Table S3). The significantly enriched KEGG pathways mainly included PI3K–Akt signaling pathway and Calcium signaling pathway (Fig.2b and Additional file 4: Table S4). Previous studies documented that the PI3K/Akt signaling pathway participate in regulating growth hormone secretion[84] and Calcium signaling pathway involved in controlling excitability of anterior pituitary cell[85]. These findings suggest that miRNA in exosomes of anterior pituitary could regulate intracellular signal transduction, catabolism and development.

Overview of lncRNA deep sequencing data in exosomes and bioinformatics analysis

LncRNA is a class of RNA molecules with transcript length over 200nt and do not encode proteins. According to its characteristics, we set filter criteria and counted the number of transcripts screened per step (Additional Fig.3a). For lncRNAs prediction, CPC and CNCI were used for potential coding ability detection, and PFAM, a protein database, was used for protein annotation information analysis were used for potential coding ability detection (Additional Fig.3b). Resultantly, 15545 novel lncRNAs and 687 annotated lncRNAs (Additional file 5: Table S5) were identified respectively. We performed statistics on different types of lncRNA mainly for lincRNA, anti-sense_lncRNA and intronic_lncRNA. The results

showed that the percentage of intronic_lncRNA was the highest (Fig.3a). The structure and sequence conservation of lncRNA and mRNA were also compared and analyzed. We found that lncRNAs were with shorter length in transcript (Additional Fig.3c) and their genes tend to contain fewer exons (Fig.3b). Most of the mRNA had longer open reading frames than lncRNA (Additional Fig.3d). The transcript expression level of lncRNA was higher than miRNA (Additional Fig. 3e) and we got same perception by comparing the FPKM of exosomes from the different samples (Additional Fig. 3f). We investigated the possible functions of the lncRNAs by searching for protein-coding genes 100 kb upstream and downstream of all identified lncRNAs to predict the potential cis-regulatory targets of lncRNAs. A total of 57087 protein-coding genes were closest to the 9524 lncRNAs. A number of lncRNAs were detected co-expression with pituitary-specific genes including growth hormone 1 (GH1), growth hormone releasing hormone receptor (GHRHR), prolactin releasing hormone receptor (PRLHR), follicle stimulating hormone subunit beta (FSHB), luteinizing hormone subunit beta (LHB) (Fig.3c). Otherwise, some lncRNAs could co-expressed with genes involved in exosome marker protein, protein transport and exosome docking such as CD63, CD81, TSG101, Rab27A, Rab27B, UBL3. Our GO annotation indicated that the predicted target genes of lncRNA were significantly enriched in cellular component biogenesis, organelle organization, RNA biosynthetic process, the cellular component of nucleus and organelle part, organic cyclic compound binding, nucleotide binding and small molecule binding (Fig.3e and Additional file 6:Table S6). The KEGG pathways enriched in Ras signaling pathway, Hippo signaling pathway and Cell cycle (Fig.3f and Additional file 7:Table S7). Hippo signaling pathway could play a role in pituitary development and regulating pituitary stem cells[86]. Ras signaling pathway could regulate pituitary cell-specific gene expression[87]. These data indicate that lncRNA in exosomes of anterior pituitary could participate in the development of pituitary and RNA biosynthetic process.

Overview of circRNA deep sequencing data in exosomes and bioinformatics analysis

After evaluated the data output quality, we obtained 494 novel circRNAs (Additional file 8:Table S8) and then counted the length distribution and the source of circRNA for all samples (Fig.4a). It showed that the length of circRNAs are mostly scattered in the area less than 10000nt and the source of circRNA mostly from intergenic area compared with exon and intron area (Fig.4b). The expression levels of all circRNAs were statistically analyzed and normalized by TPM (Fig.4c). TPM density distribution allows overall inspection of gene expression patterns in samples and the results showed there are lots of overlap which means consistency between samples[88]. We then constructed a circRNA-miRNA co-expression network based on the RNA-seq results. CircRNA could inhibit the function of miRNA by combining with miRNA[89]. So the analysis of miRNA binding sites on the identified circRNA helps further study for the function of circRNA. Then we used miRanda software to predict the miRNA binding site of the cleaved circRNA and focused on the circRNAs which were combined with highly expressed miRNAs in pituitary and exosomes from anterior pituitary. A network map was constructed containing 39 circRNAs, 8 miRNAs and 49 relationships (Fig.4d). In order to explore the potential functions of the circRNAs in exosomes from anterior pituitary, we performed GO and KEGG pathway enrichment analysis. The results showed that the enriched GO terms were mainly associated with metabolic process, cellular biosynthetic process, binding and transferase activity (Fig.4e and Additional file 9:Table S9) and the KEGG pathways were mainly

enriched in the Wnt signaling pathway, regulation of actin cytoskeleton, protein processing in endoplasmic reticulum and phagosome (Fig.4f and Additional file 10:Table S10). These findings suggest that circRNA in exosomes of anterior pituitary could regulate the cellular metabolic and biosynthetic process.

Analysis of crosstalk among lncRNA-miRNA-mRNA in exosomes

Recent studies suggested that lncRNAs could function as endogenous miRNA sponges to prevent miRNA from binding to reduce the regulatory effect of miRNAs on their target mRNA[90-92]. To further analyze the crosstalk between lncRNA, miRNA and mRNA, we predicted the interaction of them and further focused on the competitive endogenous RNAs (ceRNAs) relative with pituitary function. Then make the network that 97 lncRNAs could sponge 11 miRNAs to regulate 10 pituitary-specific genes including GH1, GHRHR, PRLHR, FSHB, LHB, proopiomelanocortin (POMC), growth hormone receptor (GHR), prolactin receptor (PRLR), gonadotropin releasing hormone receptor (GNRHR), POU class 1 homeobox 1 (POU1F1) (Fig.5a). Moreover, we performed enrichment analysis of all of dates using the GO and KEGG analysis. GO analysis revealed 273 significantly enriched terms ($P < 0.05$, Additional file 11:Table S11) in the categories of biological process, molecular function, and cellular components and we showed a part of terms with lots of gene numbers (Fig.5b). Its annotation indicated that they participate in intracellular signal transduction, cellular component organization or biogenesis, RNA metabolic process, localization, regulation of metabolic process, binding and regulate catalytic activity which suggest that they are involved in the body's basic biological regulation. KEGG pathway analysis demonstrated that 169 terms (Additional file 12:Table S12) were enriched and we selected the top 20 (Fig.5c) in which the MAPK signaling pathway, GnRH signaling pathway and Dopaminergic synapse were involved in the regulatory function about information transfer about pituitary. These results suggest that the crosstalk among lncRNA-miRNA-mRNA could participate in pituitary signaling process.

Analysis of crosstalk among circRNA-miRNA-mRNA in exosomes

The current studies have proved that circRNAs could role as ceRNAs to compete for miRNA-binding sites to affect the function of miRNA [93, 94]. Therefore, analysis of interactions between miRNAs and circRNAs is helpful for further study. In the constructed potential circRNA-miRNA-mRNA associations, similarly we mainly concerned the ceRNAs relative to pituitary function. The resultant network was comprised of 188 edges among 11 miRNAs, 58 circRNAs and 10 pituitary-specific genes including GH1, POMC, GHR, GHRHR, PRLR, LHB, PRLHR, FSHB, GNRHR, POU1F1 (Fig.6a). In order to learn the potential functions of the associated non-coding RNA in exosomes from anterior pituitary, we conducted GO and KEGG enrichment analysis. GO analysis revealed 265 significantly enriched terms (Additional file 13: Table S13) and we showed some terms enriched a lot of gene numbers (Fig.6b). Our GO annotation indicated that they are involved in intracellular signal transduction, cellular component organization or biogenesis, transport, protein binding, hydrolase activity and phosphotransferase activity. Our KEGG pathway analysis demonstrated that 267 terms (Additional file 14: Table S14) were enriched and we showed the top 20 (Fig.6c) in which the MAPK signaling pathway, Prolactin signaling pathway, GnRH

signaling pathway, Estrogen signaling pathway, and Dopaminergic synapse are participated in the regulation of hormone secretion in the anterior pituitary. These findings suggested that the network among circRNA-miRNA-mRNA in exosomes play an important role in pituitary endocrine functions.

Discussion

Exosomes contain plentiful cargoes including proteins, lipids, and nucleic acids which are specifically sorted and packaged, and contents packed are cell type-specific[95]. More and more evidences indicate that exosomes can transfer important cargoes such as miRNA, mRNA, and proteins from cell to cell via membrane vesicle delivery, thereby being a new approach of intracellular or organ-to organ communication[96-99]. Studies have reported that exosomes can mediate the transmission of information between endothelial cells, smooth muscle cells, cardiomyocytes, stem cells and fibroblasts[100-102]. Hepatocyte-derived exosomes could act as potential biomarkers of liver disease and promote cell proliferation and liver regeneration [103, 104]. Exosomes secreted by skeletal muscle contain proteins and miRNAs that can be transferred to adjacent muscle cells[105]. Exosomes from adipose tissue could mediate activation macrophage-induced insulin resistance and regarded as the main immune regulator secreted by insulin resistance factors[106, 107]. As an important endocrine gland, if the pituitary gland produce exosome and its cargos remains unclear up to date.

Firstly exosome produced from pig pituitary was successfully separated by centrifuge procedure and identified using transmission electron microscopy and western blot detection of CD9 and CD63, followed by RNA extraction and sequence. A total of 416 miRNAs were obtained from samples, 343 of which are known miRNAs and 73 are newly predicted miRNAs. Our research group has finished revealing the expression of miRNAs in porcine anterior pituitary cells and found that miRNAs could regulate the hormone secretion from the anterior pituitary[43, 108, 109]. Interestingly, we found most of top 20 miRNAs like miR-7, miR-375, let-7a, let-7c, miR-26a, miR-30a, let-7g, miR-30d, miR-127, miR-151, miR-21, miR-149, miR-99a, miR-143 in exosome were also highly expressed in the porcine pituitary[110]. Numbers of studies also revealed several of their enrichment and function. MiR-7 is abundant in the pituitary of mouse[111], pigs[112, 113]. Research showed that miR-7 might play an important role in the HPG axis and be involved in body growth by acting on the pituitary(GHRHR) in pigs[112, 114]. MiR-375 could regulate pituitary pro-opiomelanocortin (POMC) expression[115]. Let-7f-5p was a highly expressed miRNA of let-7 family in pituitary[116]. Mir-26a plays an important role in cell cycle control by modulating protein kinase C delta[117]. MiR-200b could stimulate luteinizing hormone (LH) levels by targeting ZEB1[118]. KEGG and GO analysis suggest that miRNAs in exosomes of anterior pituitary could regulate intracellular signal transduction, phosphorylation, catabolism and development, which needs further research.

CeRNAs regulate gene expression by competitively binding to microRNA[119]. Recent studies have shown that the interaction of the miRNA seed region with mRNA is not unidirectional, but that the pool of mRNAs, lncRNA[120], circRNA[52, 53] compete for the same library of miRNA to regulate miRNA activity[121]. These ceRNAs act as molecular sponges for miRNA through their miRNA binding sites to

inhibit target genes of the respective miRNA family. Unlike miRNAs, the function of lncRNAs and circRNAs are poorly understood in pig pituitary.

Researches have shown that the anterior pituitary lncRNA of rat play an important role in hormone and reproduction development and regulation[122]. In our study, some lncRNAs could co-expression with pituitary-specific genes like GH1, GHRHR, PRLHR, FSHB, LHB. Otherwise, some lncRNAs could co-expressed with genes involved in exosome marker protein, protein transport and exosome docking such as CD63, CD81, TSG101, Rab27A, Rab27B, UBL3. The signal of a two-circRNA was found could predict tumor recurrence in clinically non-functioning pituitary adenoma[123]. Another study determined that thousands of sheep genes could express circRNAs in the pituitary gland[124]. Regarding circRNA, we determined that numerous circRNAs interact with highly expressed miRNAs both in exosomes and pituitary that are involved in the biologic functions of the pituitary gland. Our preliminary analysis also suggest that ceRNAs in exosomes from anterior pituitary may participate in cellular metabolic and biosynthetic process and the cross-talk between the mRNA, miRNA, lncRNA, and circRNA may participate in the regulation of pituitary endocrine functions and signaling process.

Conclusion

Our study is the first exploration of the expression of non-coding RNA in exosomes delivered by anterior pituitary. Importantly, miRNA, lncRNA, and circRNA of exosomes from anterior pituitary may act as novel regulators of pituitary development and endocrine regulation. These findings provided a catalog of exosomes derived from the anterior pituitary and be helpful to explore the potential function of non-coding in it.

Declarations

Ethics approval and consent to participate

All animal experimentation complied with the laboratory animal management and welfare regulations approved by the Standing Committee of Guangdong People's Congress (Guangzhou), China.

Consent for publication

Not applicable.

Availability of data and materials

The raw sequence reads have been deposited in the NCBI Sequence Read Archive. BioProject accession number is PRJNA644768. Additional datasets of this article are included within the manuscript and additional files.

Competing interests

The authors declare no conflict of interest.

Funding

The research was supported by grants from the National Key Research and Development Program of China (2016YFD0500503), Natural Science Foundation of China program (31802156), and the Key Project of Guangdong Provincial Nature Science Foundation (2018B030311015). The funders had no role in study design, sample collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

YZ, QX, JS conceived and designed the experiments; JX experiments and analyzed the data. HZ, BZ, JL, TC contributed reagents, materials, and analysis tools. JX wrote the paper. YZ revised the paper. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

Abbreviations

GH: growth hormone; PRL: prolactin; ACTH: adrenocorticotropin hormone; TSH: thyroid-stimulating hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone; miRNA: microRNA; lncRNA: long non-coding RNA; circRNA: circular RNA; ncRNA: Non-coding RNA; ceRNAs: competitive endogenous RNAs; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; GH1: growth hormone 1; GHRHR: growth hormone releasing hormone receptor; PRLHR: prolactin releasing hormone receptor; FSHB: follicle stimulating hormone subunit beta; LHB : luteinizing hormone subunit beta; POMC: proopiomelanocortin; GHR: growth hormone receptor; PRLR: prolactin receptor; GNRHR: gonadotropin releasing hormone receptor; POU1F1: POU class 1 homeobox 1

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Figures

Figure 1

Isolation and identification of exosomes from anterior pituitary of Duroc swine. a Transmission electron microscopy analysis. The arrow points to exosomes. b Size distribution analysis of exosomes. c Exosomes were confirmed by Western Blot with two positive markers CD9 and CD63

a

b

Figure 2

Overview of small RNA deep sequencing data in exosomes and analysis. a Gene ontology (GO) annotation analysis b Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis enrichment analysis of miRNA's target genes. BP = biological process; CC = cellular component; MF = molecular function

Figure 3

Overview of lncRNA deep sequencing data in exosomes and bioinformatics analysis. a lncRNA type distribution map. b Number density map of lncRNA and mRNA exon. c lncRNA-mRNA co-expression (green color and yellow color represent gene and lncRNA respectively). d GO annotation analysis. e KEGG pathway analysis enrichment analysis. BP = biological process; CC = cellular component; MF = molecular function.

Figure 4

Overview of circRNA deep sequencing data in exosomes and bioinformatics analysis. **a** The length distribution of circRNA for all samples. The x-axis length (nt) represents the length distribution of circRNA full length; the y-axis represents different samples; the z-axis count represents the number of circRNAs. **b** The source of circRNA for all samples. It shows the number of exon, intron, intergenic in the circRNAs of each sample by polar coordinate fan chart, and log2 conversion of the statistics on the coordinate axis. **c**

TPM density map. It showed consistency between samples. d The network of circRNA-miRNA co-expression (red color and blue color represent miRNA and circRNA respectively) e GO annotation analysis f KEGG pathway analysis enrichment analysis.BP = biological process; CC = cellular component; MF = molecular function.

Analysis of crosstalk among lncRNA-miRNA-mRNA in exosomes. a The ceRNA network of lncRNA, miRNA and pituitary-specific genes. b GO annotation analysis. GO analysis show significantly enriched terms (P < 0.05) in the categories of biological process, cellular components and molecular function. c KEGG enrichment analysis of top 20.

Figure 6

Analysis of crosstalk among circRNA-miRNA-mRNA in exosomes. a The ceRNA network of circRNA, miRNA and pituitary-specific genes. b GO annotation analysis. GO analysis show significantly enriched terms ($P < 0.05$) in the categories of biological process, cellular components and molecular function. c KEGG enrichment analysis of top 20. BP = biological process; CC= cellular components; MF = molecular function.

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