Analysis of Long Noncoding RNA and mRNA Profiling in Peripheral Blood Mononuclear Cells of Human Immunized With Rabies Virus Vaccine by RNA Sequencing

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Abstract

Background

Rabies is still a lethal disease caused by rabies virus (RABV), and it remains a global health threaten. The epigenetic landscape of host in response to immunization with rabies virus vaccine is not yet elucidated. Evidences reveal that long noncoding RNA (lncRNA) play an important role for control of viral infection, but the expression profile of lncRNA in human immunized with rabies virus vaccine remains unclear.

Methods

lncRNA and mRNA profiles were investigated in four volunteers vaccinated with RABV vaccine by RNA sequencing.

Results

33 lnRNAs and 427 mRNAs were differentially expressed in RABV vaccine immunized volunteers. The gene oncology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to analysis the functional annotation, and the result suggested the lncRNAs involved in signaling pathways related to host immune induced by the RABV vaccine.

Conclusions

To the best of our knowledge, it is the first time to report the transcriptomic landscape of lncRNAs in human immunized with RABV vaccine. Our study mimics the host response of human to RABV infection and suggests that lncRNAs might be of a potential therapeutic target in anti-rabies virus strategies.

Background

Rabies is not only one of the oldest diseases in human history, also is one of the deadliest zoonosis disease[1]. Since the clinical symptoms had developed, the mortality rate is almost 100%[2]. No matter in developing or developed country, the rabies viral (RABV) post a serious threat to the publish health [3]. Annually the more than 70000 human deaths result from the rabies viral infection and Asia is the disaster stricken area among the worldwide[4].

RABV belongs to the family *Rhabdoviridae*, genus *Lyssavirus*, and it is one of the most important zoonosis affecting the central nervous of mammals [5]. RABV has an approximately 12 kb negative-sense RNA genome which encodes five structural proteins: the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) [6]. Following infection, the RABV virus replicates locally in muscle tissue and then travelled within axons in the peripheral nervous system (PNS) via retrograde axonal [7]. Once the clinical symptoms of rabies had developed, it elicit neuronal dysfunction and lead to death[8].

During the infection, the host innate immune system sensed the microorganisms invasion and induced the cytokines secretion by pattern-recognition receptors (PRRs)[9, 10].The cytokines contains type I interferons (IFNα/β), inflammatory cytokines, and chemokines and so on. Here in the RABV infection, the IFN-mediated immune response is the most important way in antiviral state [11, 12]. The type I IFN-IFNR signal way activates Janus-activated kinase/signal transducer(JAK/STAT)[13], result in the transcription of hundreds of IFN-stimulated genes(ISGs)[14, 15]. ISGs exert diverse antiviral effects as the effector of type I IFN response [16, 17], and inhibit the IFN mediated respond against RABV infection aggravate viral infection *in vitro* and *in vivo* [18, 19]. Although some studies demonstrated the importance of IFN mediated response against RABV infection, the mechanism by which RABV causes fatal disease remains unclear.

RABV infection can induce the host immune system responds, and the long noncoding RNAs (lncRNA) affect many cellular processes as an effector in the immune system during the infection[20, 21], such as differentiation[22], apoptosis[23], development[24], and immune responses[25]. Studies had reported that viral infections such as influenza (IAV)[26], HIV[27], hepatitis B[28] could induce specific lncRNA promote or suppress antiviral responses[29]. Most ISGs and some cellular factor play a role of antiviral or proviral function and it had been shown that IFN pathway regulates the expression of some lncRNAs. Viral-induced lncRNAs should decrease the antiviral response, while cellular lncRNAs induced by infection could either induce the pathway or decrease IFN synthesis and signaling to control the duration and the strength of the antiviral response[30–32]. Hironori et al., report that the lncRNA#32 silence could reduce ISG expression, exerted anti-virus infection which suggest lncRNA#32 positively regulates the host antiviral response[33].However, little is known about lncRNA expression profile after RABV infection and their regulating role in innate immune against RABV. In our previous work, we first had analysis the expression profiles of lncRNAs and mRNAs in brains of mice infected by rabies virus and found some differentially expressed lncRNA and mRNA[34]. What's more, we are studying the mechanism of some selected lncRNA anti-viral function.

To investigate how the lncRNA respond during the RABV infection, we use a commercial RABV vaccine to develop a model to simulate the viral induced immune respond. We analyzed the lncRNA expression profile in peripheral blood mononuclear cells (PBMCs) of volunteers after RABV vaccine immunized by RNA-SEq. The data reveal that RABV vaccine induced significant expression of lncRNA and mRNA. Gene ontology (GO) and KEGG analysis suggested lncRNA involve in the host immune response induce by the RABV vaccine. To our knowledge, this is the first study to profile the lncRNA expression induced by the RABV vaccine and the finding base on the RABV vaccine would be useful for the understanding the infection between RABV and host immunity.

Methods

Experimental design

All the volunteers enrolled for this study had all signed the Informed Consent, and the study was performed in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of Yuebei People's Hospital, Shantou University Medical College, Shaoguan, China.

All the four healthy volunteers with no rabies virus vaccine history, were recruited to receive three doses of Rabies Vaccine (Vero Cell) for Human Use (China Food and Drug Admistration approval number: S20120016) (Changsheng Bio-technology Co., Ltd). The vaccine is a fixed rabies vaccine virus aG strain, which was inoculated into Vero cells and culture in a bioreactor microcarries. After culture, the virus solution was harvested, inactivated, purified and lyophilized with appropriate stabilizers. The titer of the rabies vaccine is not lower than 2.5 IU. The vaccinate procedure was followed the pre-exposure procedure according to the manufacturer's instructions. It contained 3 injections: 1 dose was injected on 0 days, 7 days, 21 days (Fig. 1). And 10 ml peripheral blood samples were obtained from the volunteers at day 0 before vaccinated and the 28-days, the PBMCs had been separated immediately using Ficoll-Paque (GE Healthcare) gradient centrifugation for further sequence.

Total Rna Extraction

Total RNA was extracted from PBMC according to the manufacturer's protocol (TRIzol™ Reagent, Invitrogen). The quantity and purity of total RNA were evaluated by Nanodrop 2000. The ratio of A260/A280 should be from 1.8 to 2.0. RNA integrity was analyzed by the Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

High Throughput Sequencing

Ribo-Zero rRNA removal kit (Epicentre, USA) was used to remove ribosomal RNA. The rRNA-depleted RNA with NEBNext® Ultra™ Directional RNA Library Prep Kit (NEB, USA) for Illumina was used for preparation of the RNA libraries. Library sequencing was performed on an Illumina HiSeq 2000 platform with 150 bp read length in ShenZhen Realomics Inc.

Bioinformatic Analysis

With the Illumina HiSeq 2000 platform, The sequence raw data was filtered by the low quality reads, like the adaptors, low-quality reads and poly-N reads, to obtain clean data using the SOAPnuke. The filtered contain removal of adapter reads; removal of low-quality reads (the base number of sQ < = 5 accounts for more than 50% of the whole read); removal of the base information that undetermined ratio of reads is more than 10%. The QC checks is performed on the clean data to determine the suitable data for subsequent analysis, such as the Q20, Q30, and GC information. The filtered reads were mapped to the human reference genome by Tophat2 (Version 2.1.1).Then the mapped reads were assembled by reference annotation with transcripts method Cufflink (Version 2.2.1) [35]. LncRNA transcripts exceed 200nt and do not encode proteins. According to the characteristics of the lncRNA structure and the functional characteristics, the candidate novel lncRNA is obtained should accord with the followed requirement: (1) exon number ≥ 2, (2) length > 200 nt, (3) FPKM ≥ 0.5, (4) without coding capacity, (5) don't overlap with mRNA or annotated lncRNA. Coding ability was predicted using coding-non-coding-index (CNCI)[36], coding potential calculator (CPC)[37] and coding-potential assessment tool (CPAT)[38]. Cuffdiff (version 2.2.1) was used to analysis the expression, the function analysis was performed with the GO (www.geneontology.org) and KEGG (http://www.genome.ad.jp/kegg/). GO terms analyses were performed to identify biological processes enriched in neighboring genes of predicted co-localized lncRNA. KEGG was used to analyze the enriched pathways of co-expressed genes for the predicted co-localized lncRNA. Q values < 0.05 were considered as significantly enriched pathways.

Quantitative Real-time Pcr Validation

The surplus of the RNA sample for the RNA-Seq was used for quantitative real-time polymerase chain reaction (RT-qPCR) validation. RT-qPCR was used the Luna® Universal One-Step RT-qPCR Kit (NEB, USA) in LightCycler480 II (Roche, USA) according to the manufacturer's instructions. The reaction conditions were set as follows: reverse transcription at 55℃ for 10 min, initial denaturation at 95℃ for 1 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 72℃ for 30 s. The specificity of the amplified products was evaluated by using dissociation curves. The relative expression levels of lncRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method and were normalized by GADPH. The tests were triplicated.

Statistical analysis

Data were analyzed using Student's t-test or one-way ANOVA followed by Dunnett's multiple comparison test (compare post-immunized groups to the pre-immunized group). All data are demonstrated as the means ± S.D. (*P< 0.05, **P< 0.01, ***P< 0.001). For correlation studies, a two-tailed non-parametric Spearman analysis was used. $P \leq 0.05$ were considered as significant.

Results

Identification of differentially expressed lncRNAs in PBMCs of human immunized with rabies virus vaccine by RNA-seq

To investigate the lncRNA expression profile in the volunteers induced by the RABV vaccine, high throughput RNA sequencing was performed on the PBMC of volunteers immunized with RABV vaccine. We sequenced 8 rRNA-deprived total RNA samples obtained from 4 volunteers pre- and post-immunized with RABV

vaccine. Average 86 million raw reads were produced from Illumina HiSeq platform. By the bioinformatic analysis, the raw data was filter out for further analysis.

Because of the specific structure and non-coding characteristics of lncRNA, 5-step scanned was used to analysis the transcripts to identify the annotated lncRNA and novel lncRNA. Finally 558 novel lncRNA were assembled by Cuffilink (Fig. 2A). CNCI, CPC and CPAT were used to evaluate the coding capacity of transcripts (Fig. 2B). Moreover, with the relative location to the coding genes, the lncRNA identified were divided into five classifications include intergenic lncRNA (74.55%), intronic lncRNA (10.22%), antisense lncRNA (6.63%), enhancer lncRNA (3.41%) and bidirectional lncRNA (5.20%) (Fig. 2C).

The lncRNA expression profiles in eight sample of four volunteers pre- and post-immunized with RABV vaccine were analyzed with hierarchical clustering. The result showed that lncRNA expression profiles were significantly modified after RABV vaccine immunized (Fig. 3A). There was 33 lncRNA were expressed significant different, including 1 upregulated and 32 downregulated (Fig. 3B). The upregulated lncRNA was ENSG00000254162 with a fold change (FC) of 4.38 compared to the post-immunized and the downregulated lncRNA had a FC range 1.75 to 7.07 (Table 1). All the differentially expressed lncRNA were listed in **Table 1**. The number distribution of lncRNA in all chromosomes was display in Fig. 3C and it showed that chromosome 5 had the highest number of lncRNA, while chromosome 3, 4, 6 and so on had only one lncRNA. However, several chromosomes didn't have any significant different lncRNA like chromosome 2, 10, et.al (Fig. 3C).

Identification of differentially expressed mRNAs in PBMCs of human immunized with rabies virus vaccine by RNA-seq

We examined the different expression of mRNA after the RABV vaccine immunized, and significant difference expression of mRNA induced by RABV vaccine was showed by hierarchical cluster analysis (Fig. 4). In our study, 427 mRNA were differentially expressed after RABV vaccine immunized, including 321upregulated and 106 downregulated (Fig. 4B). In the different expression genes, the most upregulated one was HSPA1B (FC = 14.84) and the top 3 downregulated were CLC (FC = 27.00), DEFA3 (FC = 15.54) and CAMP (FC = 11.96). The top 20 differentially expressed genes were listed in Table 2. Unlike the lncRNA, the mRNA distributed to all the chromosome but were not equally scattered among chromosomes. The chromosome 1 had the most differentially expressed mRNAs and chromosome 14 and 18 had the least numbers (Fig. 4C).

Table 1. Differentially expressed lncRNAs in peripheral blood mononuclear cells of human immunized by RABV vaccine by RNA sequencing.

Gene_id	Isoform_status	Post-immunized	Pre-immunized	log2FoldChange	p_value	q_value	Dysregulated	FC
ENSG00000245164	lncRNA_annotated	5.34448	9.3865	-0.81254	0.0002	0.020468	Down	1.756297
XLOC_042681	lncRNA_Novel	2.31781	4.21036	-0.86119	5.00E-05	0.006587	Down	1.81653
ENSG00000215458	lncRNA_annotated	6.22621	11.3207	-0.86254	5.00E-05	0.006587	Down	1.818237
ENSG00000261490	lncRNA_annotated	3.0043	5.63895	-0.9084	0.00045	0.038471	Down	1.876961
ENSG00000269821	lncRNA_annotated	1.20099	2.28741	-0.92949	5.00E-05	0.006587	Down	1.904599
XLOC_051604	lncRNA_Novel	2.42931	4.71665	-0.95722	5.00E-05	0.006587	Down	1.94156
ENSG00000281103	lncRNA_annotated	4.09089	8.10076	-0.98564	0.0001	0.011848	Down	1.980194
ENSG00000266962	lncRNA_annotated	6.01747	12.0116	-0.99721	0.0005	0.041152	Down	1.996129
XLOC_018471	lncRNA_Novel	0.977134	1.95301	-0.99907	0.00015	0.016397	Down	1.998714
XLOC_024827	lncRNA_Novel	0.902814	1.94629	-1.10822	5.00E-05	0.006587	Down	2.155795
ENSG00000235314	lncRNA_annotated	3.66534	8.05664	-1.13623	0.00055	0.044236	Down	2.198059
XLOC_056429	lncRNA_Novel	1.21387	2.79502	-1.20324	0.0005	0.041152	Down	2.302562
XLOC_050760	lncRNA_Novel	2.77627	6.39987	-1.2049	5.00E-05	0.006587	Down	2.305213
XLOC_056637	lncRNA_Novel	0.509636	1.20423	-1.24057	5.00E-05	0.006587	Down	2.362919
XLOC_049174	lncRNA_Novel	0.774507	1.85106	-1.257	5.00E-05	0.006587	Down	2.389982
XLOC_041020	lncRNA_Novel	1.62414	3.8977	-1.26295	5.00E-05	0.006587	Down	2.39986
XLOC_059380	lncRNA_Novel	0.770727	1.85765	-1.26919	0.00045	0.038471	Down	2.410262
XLOC_062407	lncRNA_Novel	0.472431	1.20236	-1.3477	0.0002	0.020468	Down	2.545061
XLOC_023926	lncRNA_Novel	0.560945	1.42784	-1.3479	0.00065	0.049892	Down	2.545413
XLOC_016347	lncRNA_Novel	0.82775	2.12904	-1.36294	5.00E-05	0.006587	Down	2.572088
XLOC_051558	lncRNA_Novel	0.922673	2.38183	-1.36818	5.00E-05	0.006587	Down	2.581447
ENSG00000230733	lncRNA_annotated	0.824332	2.15159	-1.38411	0.00025	0.024656	Down	2.610109
XLOC_051602	lncRNA_Novel	0.684579	1.84391	-1.42948	5.00E-05	0.006587	Down	2.693496
XLOC_051172	lncRNA_Novel	0.654121	1.98247	-1.59967	5.00E-05	0.006587	Down	3.03074
XLOC_066116	lncRNA_Novel	0.83044	2.59132	-1.64174	5.00E-05	0.006587	Down	3.12042
ENSG00000214894	lncRNA_annotated	1.09692	3.73051	-1.76592	5.00E-05	0.006587	Down	3.400908
XLOC_003374	lncRNA_Novel	1.10497	3.8463	-1.79947	5.00E-05	0.006587	Down	3.480923
XLOC_005414	lncRNA_Novel	0.533678	1.92461	-1.85053	0.0002	0.020468	Down	3.606326
XLOC_062716	lncRNA_Novel	0.399621	1.84311	-2.20543	5.00E-05	0.006587	Down	4.61212
XLOC_005417	lncRNA_Novel	0.363757	1.69681	-2.22177	5.00E-05	0.006587	Down	4.664654
ENSG00000197182	lncRNA_annotated	0.408312	1.96928	-2.26992	0.0004	0.035132	Down	4.822964
XLOC_000161	lncRNA_Novel	0.492994	3.48758	-2.82259	5.00E-05	0.006587	Down	7.074313
ENSG00000254162	lncRNA_annotated	3.30507	0.75435	2.13138	5.00E-	0.006587	Up	4.381364

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Table 2. Differentially expressed mRNAs in peripheral blood mononuclear cells of human immunized by RABV vaccine by RNA sequencing

Gene_id	Gene	Isoform_status	Post-immunized	Pre-immunized	log2FoldChange	p_value	q_value	Dysregulated	FC
ENSG00000105205	CLC	mRNA	1.33294	35.9944	-4.75509	5.00E-05	0.006587	Down	27.00379
ENSG00000239839	DEFA3	mRNA	4.43212	68.8862	-3.95815	5.00E-05	0.006587	Down	15.54254
ENSG00000204388	HSPA1B	mRNA	78.3372	5.27778	3.89169	5.00E-05	0.006587	Up	14.84279
ENSG00000164047	CAMP	mRNA	0.592677	7.0903	-3.58053	0.00065	0.049892	Down	11.96319
ENSG00000125740	FOSB	mRNA	96.1971	11.1305	3.11147	5.00E-05	0.006587	Up	8.642628
ENSG00000162772	ATF3	mRNA	23.1743	3.18224	2.86441	5.00E-05	0.006587	Up	7.28238
ENSG00000111537	IFNG	mRNA	11.2211	1.54555	2.86002	0.0002	0.020468	Up	7.260254
ENSG00000276085	CCL3L3	mRNA	51.5995	7.47768	2.7867	5.00E-05	0.006587	Up	6.900496
ENSG00000090104	RGS1	mRNA	52.4469	7.78688	2.75174	5.00E-05	0.006587	Up	6.73529
ENSG00000277632	CCL3	mRNA	80.3514	12.1697	2.72303	5.00E-05	0.006587	Up	6.602581
ENSG00000141682	PMAIP1	mRNA	49.184	8.15661	2.59215	5.00E-05	0.006587	Up	6.029967
ENSG00000125538	IL1B	mRNA	62.8604	10.7286	2.55069	5.00E-05	0.006587	Up	5.859144
ENSG00000118503	TNFAIP3	mRNA	263.218	50.6895	2.3765	5.00E-05	0.006587	Up	5.192754
ENSG00000185022	MAFF	mRNA	32.1323	6.22367	2.36818	5.00E-05	0.006587	Up	5.162894
ENSG00000255398	HCAR3	mRNA	14.3272	2.78187	2.36463	5.00E-05	0.006587	Up	5.150206
ENSG00000114315	HES1	mRNA	2.92857	0.57765	2.34193	0.00015	0.016397	Up	5.069804
ENSG00000110848	CD69	mRNA	219.951	43.9565	2.32303	5.00E-05	0.006587	Up	5.00382
ENSG00000169429	CXCL8	mRNA	24.4288	4.93953	2.30614	5.00E-05	0.006587	Up	4.945581
ENSG00000143507	DUSP10	mRNA	35.2674	7.27819	2.27668	5.00E-05	0.006587	Up	4.845616
ENSG00000112149	CD83	mRNA	62.9776	13.0436	2.2715	5.00E-05	0.006587	Up	4.828249

Genomic Features Of Lncrnas And Mrnas

To know more about the genomic features of the lncRNA and mRNA in volunteers, we systematically analyzed the feature of lncRNA in this study and compared them with protein-coding genes. The result show that the average expression level of lncRNA were lower than mRNAs (Fig. 5A). The exons number and the lengths of lncRNA was also less and shorter than that of mRNAs (Fig. 5B, C) Furthermore, most of the mRNAs had a longer ORFs than the lncRNA (Fig. 5D).

Functional Prediction Of Rabv Vaccine Induced Co-localized Lncrnas

To better understand the functions of differentially expressed co-localized lncRNA in RABV vaccine immunized volunteers, GO term and KEGG pathway analysis was performed. The GO analysis result reveal that the genes colocate with differentially expressed lncRNA were highly enriched in biological

processes like extracellular vesicular exosome, extracellular organelle and extracellular membrane-bounded organelle process. The top 20 GO terms are presented (Fig. 6A).

What's more, KEGG was also used to investigate the involved biological pathways of the differentially expressed lncRNA. The top20 significant enriched pathways terms exhibited in Fig. 6B. Some infectious disease pathways were shown, such Chagas disease (American trypanosomiasis), malaria, tuberculosis, herpes simplex infection, hepatitis C and measles (Fig. 6B). These finding suggested that lncRNA regulate the infectious process after RABV vaccine immunized.

Validation Of Selected Differentially Expressed Lncrnas By Rt-qpcr

To valiate the rabies vaccine induced lncRNA expression changes detected by RNA-seq, five downregulated expressed lncRNA (XLOC_005414, XLOC_062716, XLOC_005417, ENSG00000197182, XLOC_000161) and one upregulated expressed lncRNA (ENSG00000254162) were selected to validate by using RT-qPCR. The results were shown in Fig. 7. Both the results of RT-qPCR were similar to the RNA-seq result, which implicated the good reliability and reproducibility of the lncRNA changes determined by RNA-seq.

Discussion

Rabies is one of the most deadly infectious diseases and was fatal once the clinical symptoms developed, and it remains a threat to public health. Currently, without certain cure, rabies can be prevented by rabies immune globulin (RIG) or rabies vaccine injection once a bite or exposure to saliva from an infected animal. As a result, annually almost 59,000 global human deaths caused by rabies and estimated 15 million people receive post-exposure prophylaxis annually for exposures [39]. Pre-exposure prophylaxis (PEP) protect the high-risk group with the presence of rabies-specific virus-neutralizing antibodies (VNAs). Unfortunately, the pre-exposure prophylaxis or post-exposure prophylaxis was not only need multiple injections but also time-consuming and expensive [40]. Now, there is an urgent need to develop a new biologicals to potentially replace existed prophylaxis with cost-effective alternatives to the human rabies elimination.

During the infection, the viral induce the host innate immune system and secretion lots cytokines, chemokine like type I IFN [9, 10]. And the type I IFN active JAK/STAT signaling pathway result in the expression of ISGs, which had diverse antiviral functions [13–15]. A important feature of the RABV is that the RABV can replicate in the central nervous system (CNS) where was an immunologically privileged area of the host [41]. Therefore, when the RABV infected like bites or scratches, the RABV G protein binds to the nicotinic acetylcholine receptor and later enter the CNS. When the RABV exposed to the innate and adaptive immune responses, it induced some molecules and chemokines, like NF-κB, type I IFN-regulated responses and toll-like receptors (TLR) to against the elimination [42, 43] [44]. Though lots efforts had done to investigate the RABV biology and anti-RABV immune response, the mechanism of how the RABV escape the elimination in the host immune response is still need deep-going.

In our previous work, we found that the RABV infection can significantly change the protein-coding profile of host cell *in vitro* by RNA-sequencing, and identified some genes that function against viral replication, such as ISG15 and UBA7 [45, 46]. What's more, we also investigated the host immune response during the RABV infection in a mouse model and found something interesting (unpublished data), but how the human host immune response during the infection is still nuclear. Raising data had suggested that lncRNA involve in modulated many biological process like gene epigenetic modulation, protein scaffolding, cell development and more [47]. LncRNAs also have been implicated in the pathogenesis and response to bacterial and viral infections. Reports had reveal that HIV[27], SARS-CoV[48] and some viral can induce lncRNA differentially expressed. On the other way out, the viral-induced IFN pathway can regulate the expression of several lncRNA [49]. Accumulating evidences supported that many viral infections induced specific lncRNA which in turn play an antiviral role in host immune responses [21, 50]. Though cellular lncRNA were reported in many virus infections, i.e. enterovirus, influenza, hepatitis B and C viruses, the role of lncRNA in the RABV infection remains unclear.

Here we analysis the expression profile of lncRNA and mRNA in PBMC after RABV vaccine immunized. A total of 33 lnRNA and 427 mRNA were differentially expressed in RABV vaccine immunized volunteers. In the lncRNA, only one annotated lncRNA ENSG00000254162 was upregulated with a FC of 4.38 and the other 32 lncRNA were downregulated with a FC range from 1.75 to 7.07 (Fig. 3). To be noted, five of the top 6 downregulated lncRNA were novel ones (Table 1). With the Ensembl genome browser and NCBI data base, lookuped all the differentially expressed lncRNA, we found that it involved the chromatin regulatory like ENSG00000269821 and hormonal regulatory like ENSG00000266962 .However, the only one upregulated lncRNA ENSG00000254162 was short of research and whether the novel lncRNA were relevant with RABV vaccine immunization is still need to be investigate. In the 427 differential expressed mRNAs, 321 of them were upregulated and 106 were downregulated. The top 10 differential expression mRNA contained 3 down regulated mRNA and seven upregulated mRNA with a FC range from 6.60 to 27.00 (Table 2). Like DEAF3 (GENE ID: ENSG00000239839, defensin alpha 3) and CAMP (GENE ID: ENSG00000164047, cathelicidin antimicrobial peptide) are both belong to the antimicrobial peptide. They are the first line of defense against a wide range of pathogens. Data had proved that defensin can inhibit some viral infection, like HIV, influenza A virus (IAV) and so on [51–53]. Also, data showed that the mRNA expression of INF-γ and activating transcription factor 3 (ATF3) were higher than pre-immunized. What's more, ATF3 can modulate the IFN -γ expression to regulate viral infection in mice [54] [55]. It demonstrated that RABV vaccine can induce host immune against the viral infection.

Base on the unique structure and characteristic unlike protein coding genes and microRNAs, lncRNA sequences are currently uninformative for predicting function[56]. The regulation effects of lncRNA are mainly by regulating the expression of the neighboring protein coding genes [57]. Here GO term and KEGG pathway analysis was performed to investigate the functions of differentially expressed lncRNA in RABV vaccine immunized volunteers. GO terms were significantly enriched in biological processes like extracellular vesicular exosome, extracellular organelle and extracellular membrane-bounded organelle process. The involved biological pathways of the differentially expressed lncRNA analysis by KEGG contained many infectious diseases like Chagas disease (American trypanosomiasis), malaria, tuberculosis, herpes simplex infection, hepatitis C and measles. What's more, the viral induced immune responses

pathway like the toll-like receptor signaling pathway and TGF-beta signaling pathway were significant enriched pathways. Taking together, it suggested that lncRNA take part in host immune response during the RABV vaccine immunized through various pathways.

Conclusions

In a brief, it is first to reveal the lncRAN expression profile and function of differentially expressed lncRNA in RABV vaccine immunized model which mimic the RABV infection in human. In the present study, some lncRNA are identified and the data suggested lncRNA play an important role in regulating gene expression after RABV vaccine immunized and exert important biological effects. It may be very valuable for the development of new effective therapeutic drugs for rabies or provide novel strategies for vaccine designation.

Abbreviations

RABV
rabies virus; lncRNA:long noncoding RNA; GO:gene oncology; KEGG:Kyoto Encyclopedia of Genes and Genomes; JAK/STAT:Janus-activated kinase/signal transducer; ISGs:IFN-stimulated genes; PBMCs:peripheral blood mononuclear cells; RT-qPCR:quantitative real-time polymerase chain reaction; FC:fold change; RIG:rabies immune globulin; PEP:Pre-exposure prophylaxis; VNAs:virus-neutralizing antibodies; TLR:toll-like receptors; CNS:central nervous system.

Declarations

Authors' contributions

The study was designed by PZ. PZ, KH and PZ drafted the manuscript. WJ, ZP, and GH contributed to the field research. PZ and KH completed the experiment. PZ, KH, PZ, SY and XX collected and analyzed the data. All authors assisted in reviewing and editing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data analyzed during the study are included in this published article (and its supplementary information files) and are available from the included studies, which are fully referenced.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all animal experimental investigations. All animal experiments were performed following the National Institute of Health Guide for the Care and Use of Laboratory Animals, and the experimental protocols were approved by the Research Ethics Committee of Yuebei People's Hospital, Shantou University Medical College, Shaoguan, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

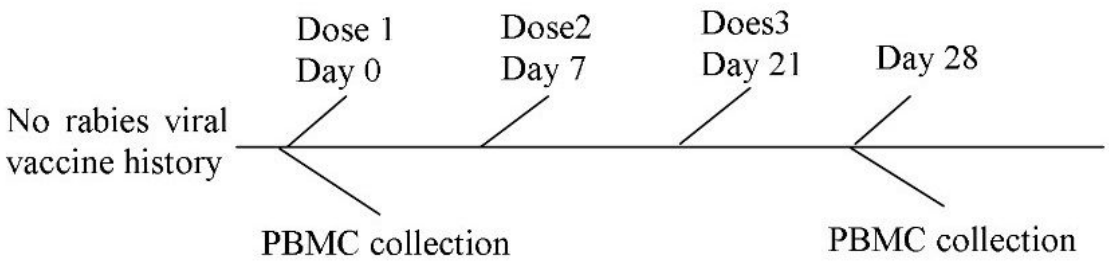

Figure 1

The immunization procedure of rabies vaccine. The immunized procedure contained 3 injections: 1 dose was injected on 0 days, 7 days, 21 days. And 10ml peripheral blood samples were obtained at day 0 before vaccinated and the 28-days, the PBMCs had been separated immediately for further sequence.

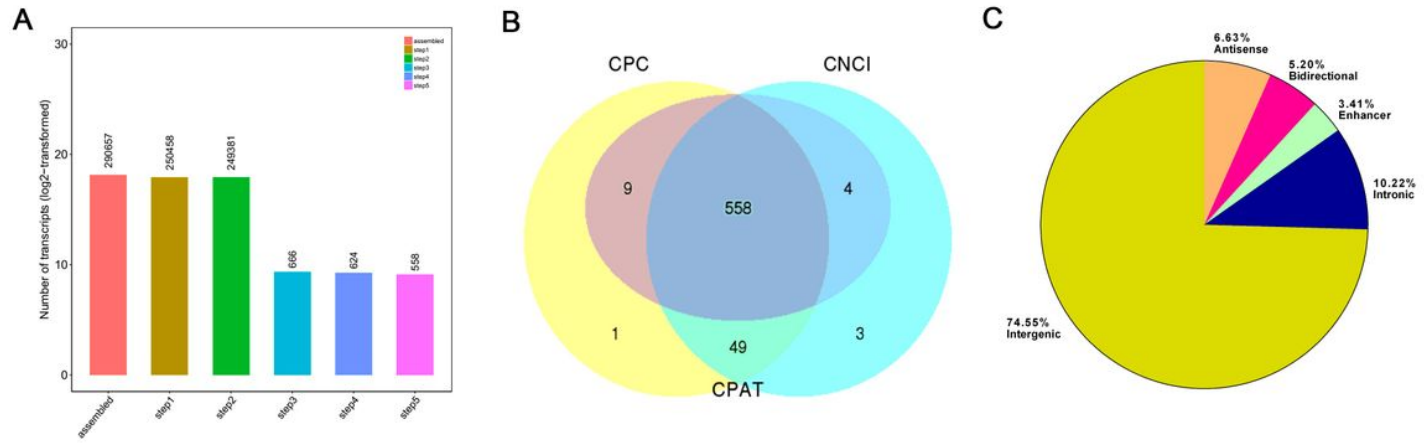

Figure 2

Identification of novel lncRNA in PBMCs after RABV vaccine immunization. (A) Screen of lncRNA in RABV vaccine immunized volunteers. (B) Evaluating the coding capacity of assembled transcripts using CNCI, CPC and CPAT. (C) Classification of lncRNA based on genomic location.

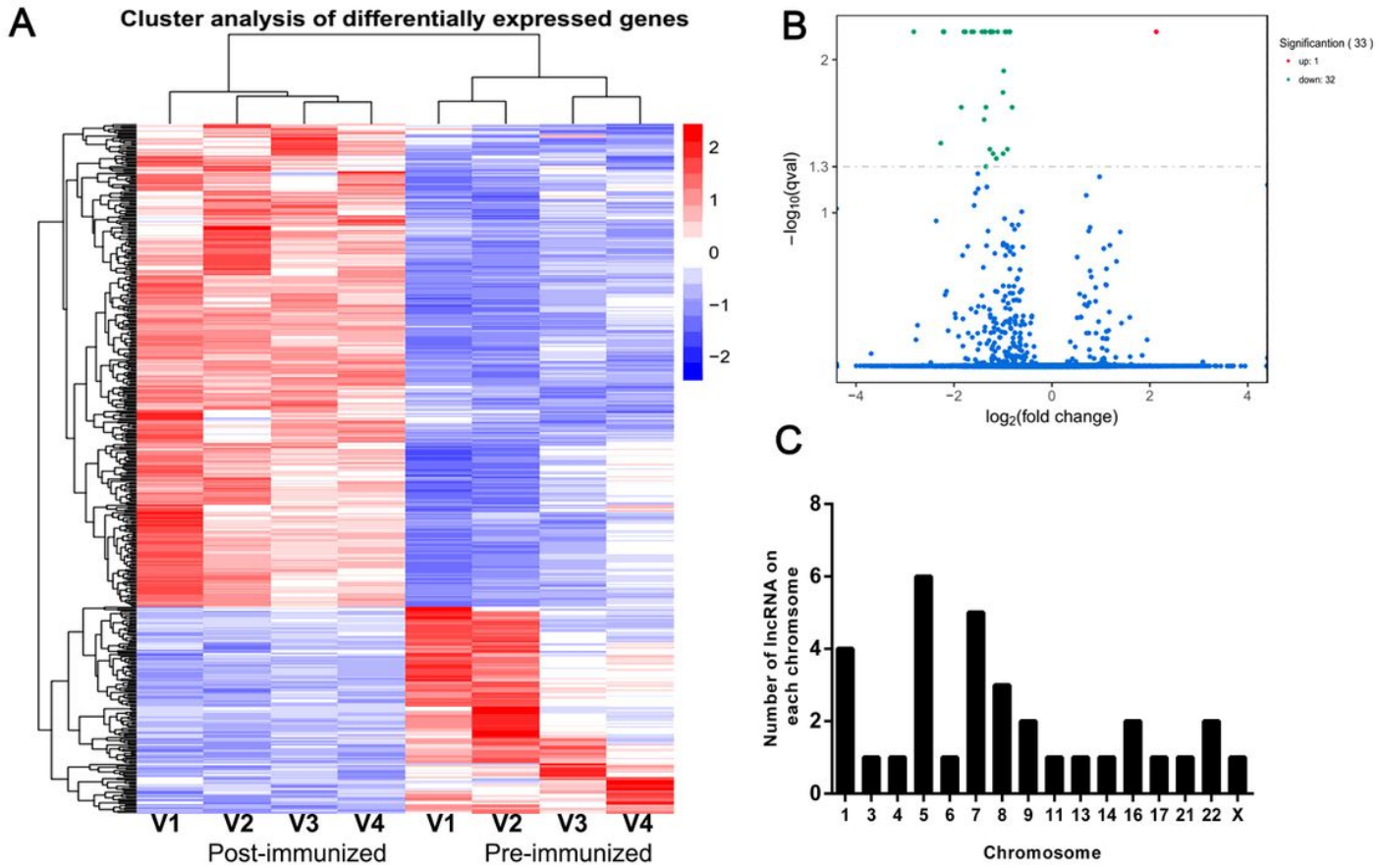

Figure 3

The expression profiles of lncRNAs in PBMCs of four volunteers pre- and post-immunization. (A) Hierarchical clustering of differentially expressed lncRNA. (B) Volcano plot of differentially expressed lncRNA in PBMC post-immunized compared with pre-immunized. (C) Distribution of differentially expressed lncRNA in each chromosome.

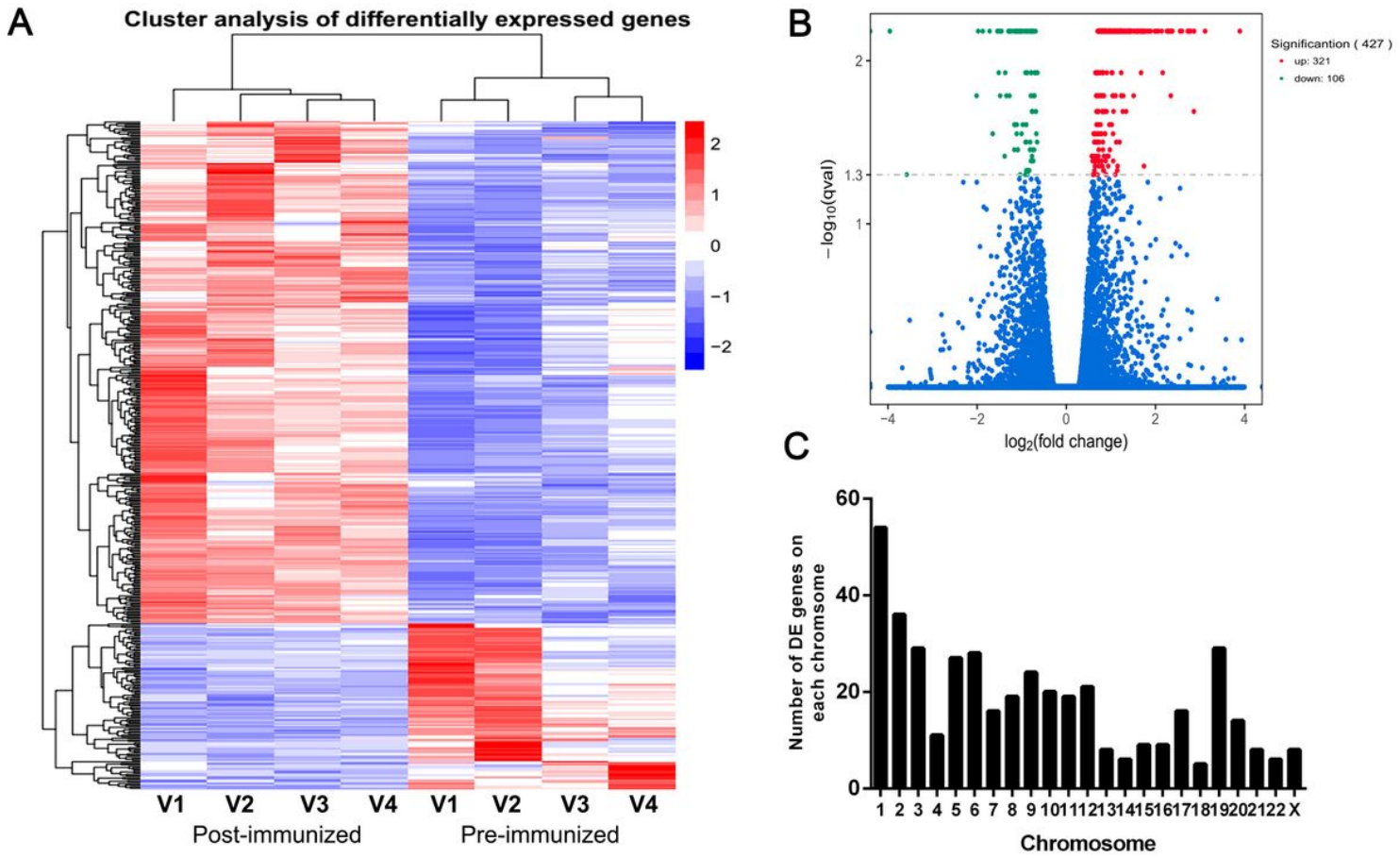

Figure 4

The expression profiles of mRNAs in PBMCs in four volunteers. (A) Hierarchical clustering of differentially expressed mRNAs. (B) Volcano plot of differentially expressed mRNAs in RABV vaccine immunized volunteers PBMC compared with pre-immunized. (C) Distribution of differentially expressed lncRNA in each chromosome.

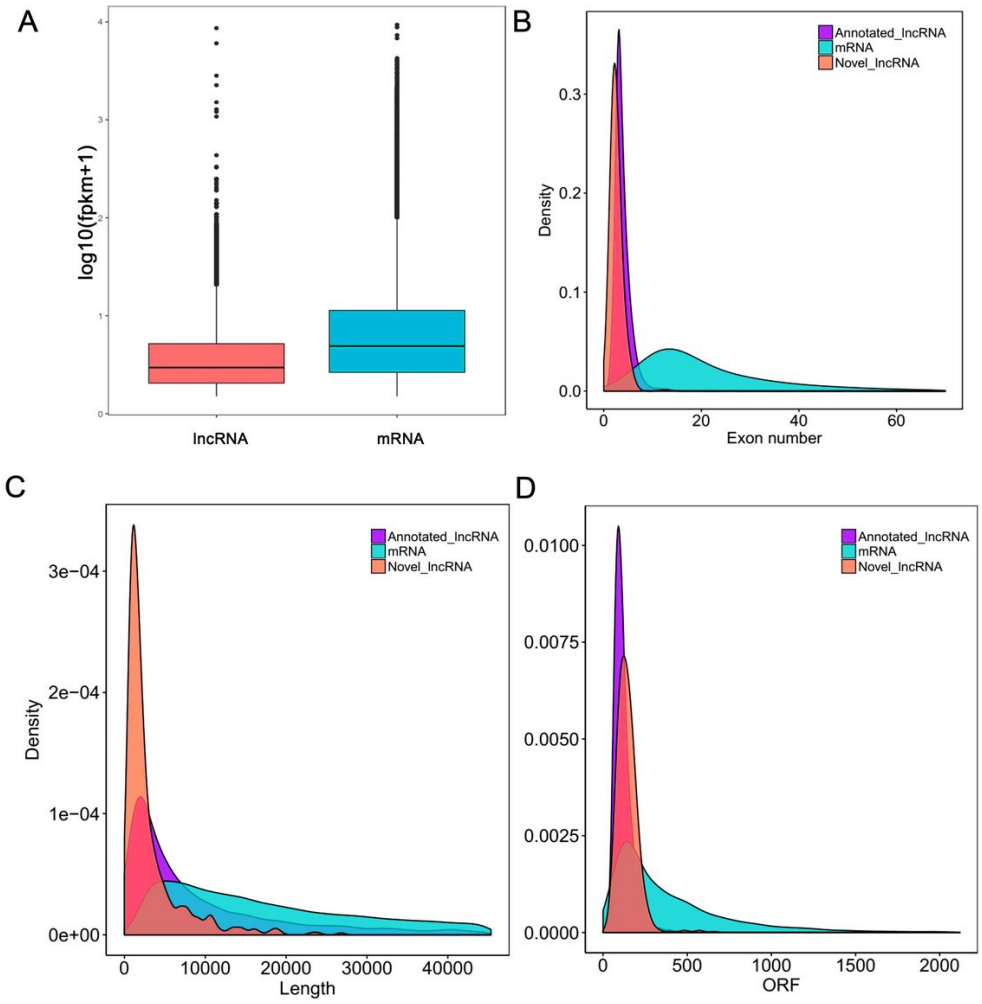

Figure 5

Genomic features of lncRNAs and mRNAs. (A)Comparison of lncRNA and mRNAs expression level. (B) Comparison of exon number between lncRNA and mRNAs (C) Length distribution of lncRNA and mRNAs. (D) Length of ORFs between lncRNA and mRNAs.

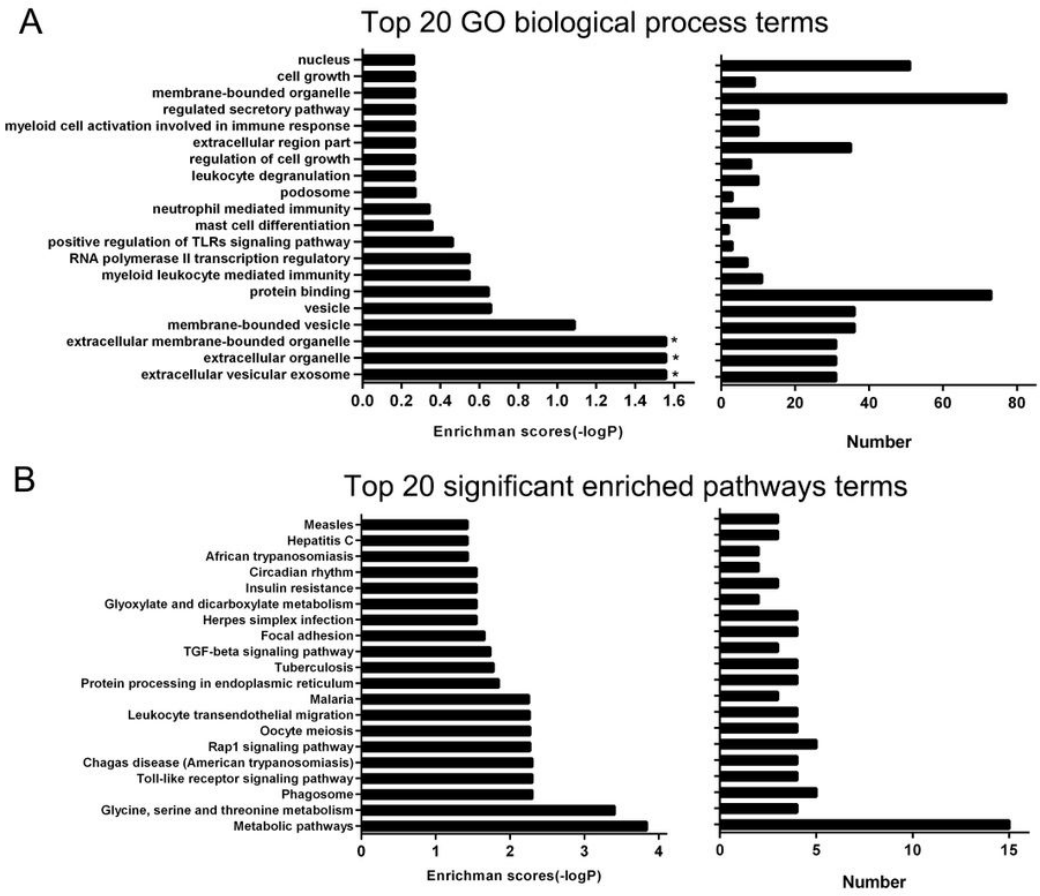

Figure 6

Go enrichment and KEGG pathway analysis of target genes of differentially expressed lncRNAs. (A) Top 20 GO biological processes enriched among target genes of differentially expressed lncRNA. (B) The top 20 pathways enriched among target genes of differentially expressed lncRNA. Right: the enrichment scores (-logP), left: the number of each.

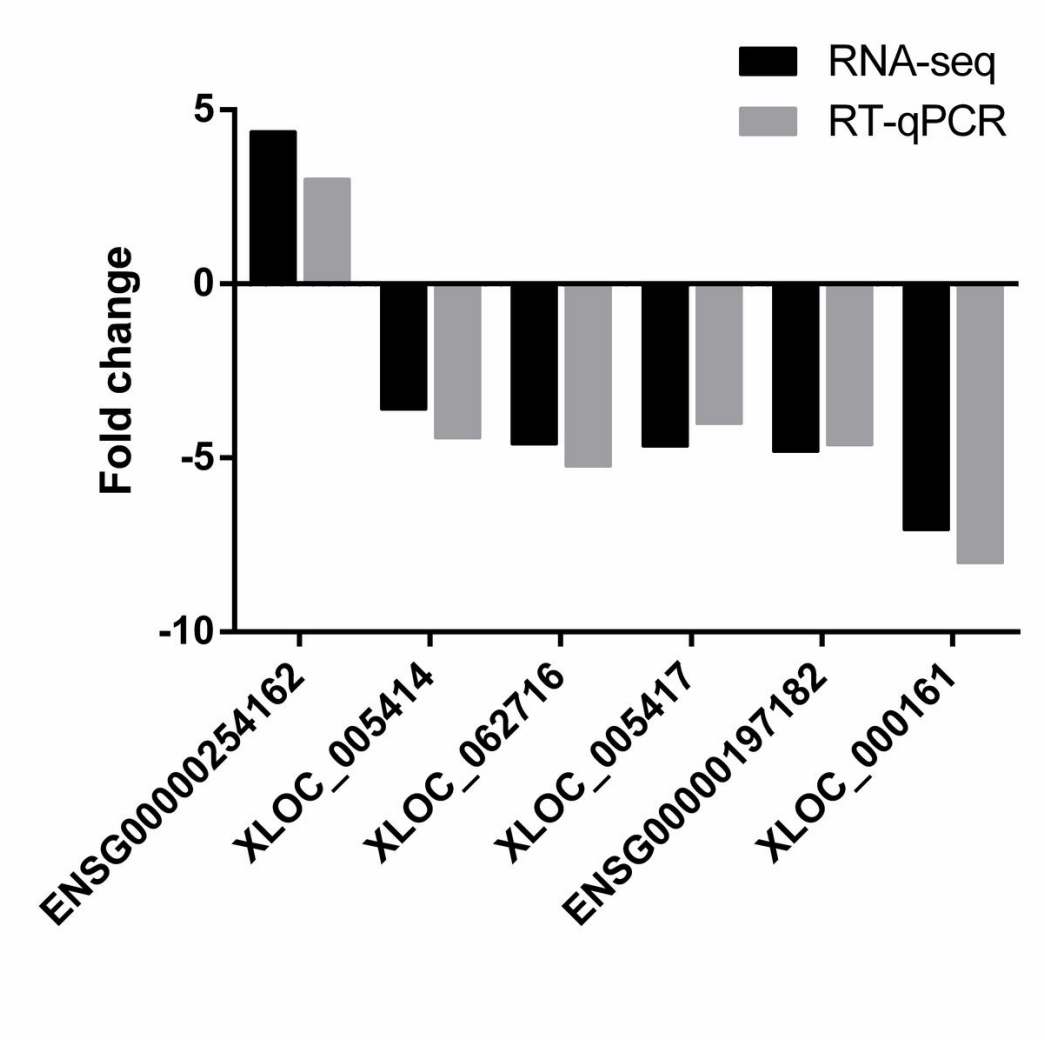

Figure 7

Validation of selected differentially expressed lncRNAs by RT-qPCR Base on the data of RAN-seq, five downregulated expressed lncRNA (XLOC_005414, XLOC_062716, XLOC_005417, ENSG00000197182, XLOC_000161) and one upregulated expressed lncRNA (ENSG00000254162) were selected to validate by using RT-qPCR.