Neuroendocrinology Letters Volume 39 No. 2 2018
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Integrated analysis of differentially expressed lncRNAs in Medial Temporal Lobe Epilepsy

Song Qiao [1], Qi Xu[2], Lin Cheng[1], Ying Zhang[3], Xiaoli Liu[1], Chaobo Zheng[1], Xiaofeng Hu[4], Linhui Chen[1]

1 Department of Neurology, Zhejiang Hospital, Hangzhou, 310000, Zhejiang, China
2 Department of Radiology, Zhejiang Hospital, Hangzhou, 310000, Zhejiang, China
3 Department of Neurology, Second Affiliated Hospital of Zhejiang University, Hangzhou, 310000, Zhejiang, China
4 Department of Cardiology, Zhejiang Hospital, Hangzhou, 310000, Zhejiang, China

Correspondence to: Linhui Chen
Department of Neurology, Zhejiang Hospital, Hangzhou, 310000, Zhejiang, China
e-mail: 465569246@qq.com

Submitted: 2017-10-31 Accepted: 2018-04-10 Published online: 2018-07-12

Key words: **Differentially expressed gene; Long non-coding RNA; Medial Temporal Lobe Epilepsy; co-expression network; bioinformatics analysis**

Neuroendocrinol Lett 2018; **39**(2):119–124 **PMID:** 30183206 NEL390210A09

Abstract

BACKGROUND: Medial temporal lobe epilepsy (MTLE) is the most frequent type of epilepsy. In recent years, the important roles of lncRNAs in regulating human diseases progression had been implicated, including in epilepsy. However, comprehensive analysis of differentially expressed lncRNAs in Medial Temporal Lobe Epilepsy was still lacking.
OBJECTIVES: The aim of this study was to explore relevant lncRNAs in MTLE.
METHODS: In present study, we analyzed a public dataset GSE25453 to identify differentially expressed lncRNAs and mRNAs in the MTLE.
RESULTS: A total of 16 lncRNAs (C6orf176, NCRNA00259, PRO1768, TTTY11, LOC149620, GDEP, LOC400891, HLA-DRB6, TTTY21, TTTY3, NBR2, TTTY1, FAM183B, C15orf51, FAM74A3, and MALAT1) were identified. LncRNA co-expression network analysis showed these lncRNAs were mainly enriched in regulating transcription, inflammatory response, DNA binding, Jak Stat Signaling Pathway, and Mapk Signaling Pathway. Meanwhile, lncRNA-mRNA-biological processes networks were also performed to evaluate the potential roles of key lncRNAs in MTLE.
CONCLUSIONS: This study will be useful to explore the potential candidate biomarkers for diagnosis, prognosis, and drug targets of MTLE.

INTRODUCTION

Epilepsy is one of the most common types of severe neurological disorders affecting more than 50 million people worldwide(BellNeligan & Sander, 2014). Medial temporal lobe epilepsy (MTLE) is the most frequent type of epilepsy (Tatum, 2012). However, the mechanism underlying MTLE progression remained largely unknown. Recent years, several groups reported a series of epilepsy-linked genes by using high-throughput techniques, such as microarray. For example, Abhilash *et al.* identified 413 different expressed genes in MTLE (Venugopal *et al.* 2012). Hengling *et al.* also collected 1,065 genes in different animal models to construct an epilepsy candidate gene database (Chen,

Xu, Du H, Yi, & Li, 2016). However, there was still an urgent need to identify novel diagnostic and prognostic biomarkers for MTLE.

More than 80% genes encode non-coding transcripts in human genome (Gascoigne *et al.* 2012). Emerging studies had shown non-coding RNAs (ncRNAs), such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), played key roles in the development of human diseases, including epilepsy. For example, Avansini *et al.* found hsa-miR-134 was a circulating biomarker for mesial temporal lobe epilepsy (Avansini *et al.* 2017). Recently, more and more attention was paid to lncRNAs, which were a class of non-coding RNAs with more than 200 nucleotides (Shi, Sun, Liu, Yao, & Song, 2013). LncRNA played its roles in human diseases by regulating expression of protein-coding genes through transcriptional, post-transcriptional, post-translational or epigenetic regulation (Wan *et al.* 2016). Of note, a few lncRNAs have already been implicated in various neural processes. For example, BACE1-AS was reported to be highly expressed during the Alzheimer's disease progression (Decourt & Sabbagh, 2011) and NEAT1 was up-regulated in Huntington's disease (RivaRatti & Venturin, 2016). Meanwhile, Lee *et al.* performed microarray analysis to identify dysregulated lncRNAs in mouse models of localization-related epilepsy (Lee *et al.* 2015). However, comprehensive analysis of differentially expressed lncRNAs in Medial Temporal Lobe Epilepsy was still lacking.

The aim of the present study was to identify differentially expressed lncRNAs in the MTLE by using a public dataset (GSE25453)(Venugopal *et al.* 2012). A series of bioinformatics analysis, including lncRNA co-expression analysis, GO and KEGG analysis were performed in this study to reveal the potential roles of differentially expressed LncRNAs (DElncs) in MTLE. This study will be useful to explore the potential candidate biomarkers for diagnosis, prognosis, and drug targets of MTLE.

MATERIALS AND METHODS

Data sources

In this study, we downloaded a public dataset GSE25453 from GEO database (GEO, http://www.ncbi.nlm.nih.gov/geo/). GSE25453 was submitted by Abhilash *et al.* and contained 10 MTLE samples and 10 control samples. T test was performed to identify differentially expressed genes in spiking zones as compared against non-spiking regions. A p-value cut-off of 0.05 and a fold value change of ≥2 was used as a filter to identify significantly expressed genes.

Co-expression network construction and analysis

In this study, the Pearson correlation coefficient of different expressed gene (DEG)-lncRNA pairs was calculated by using Co-LncRNA database (http://www.bio-bigdata.com/Co-LncRNA/). The co-expression network was established by using cytoscape software.

GO & KEGG pathway analysis

To identify functions of DEGs in MTLE, we performed GO function enrichment analysis in 3 functional ontologies: biological process (BP), cellular component (CC) and molecular function (MF). KEGG pathway enrichment analysis was also performed to identify pathways enriched in MTLE using DAVID system (https://david.ncifcrf.gov/). The p-value was calculated by hypergeometric distribution and a pathway with $p < 0.05$ was considered as significant.

Statistical analysis

The numerical data were presented as mean ± standard deviation (SD) of at least three determinations. Statistical comparisons between groups of normalized data were performed using T-test or Mann–Whitney U-test according to the test condition. A $p < 0.05$ was considered statistical significance with a 95% confidence level.

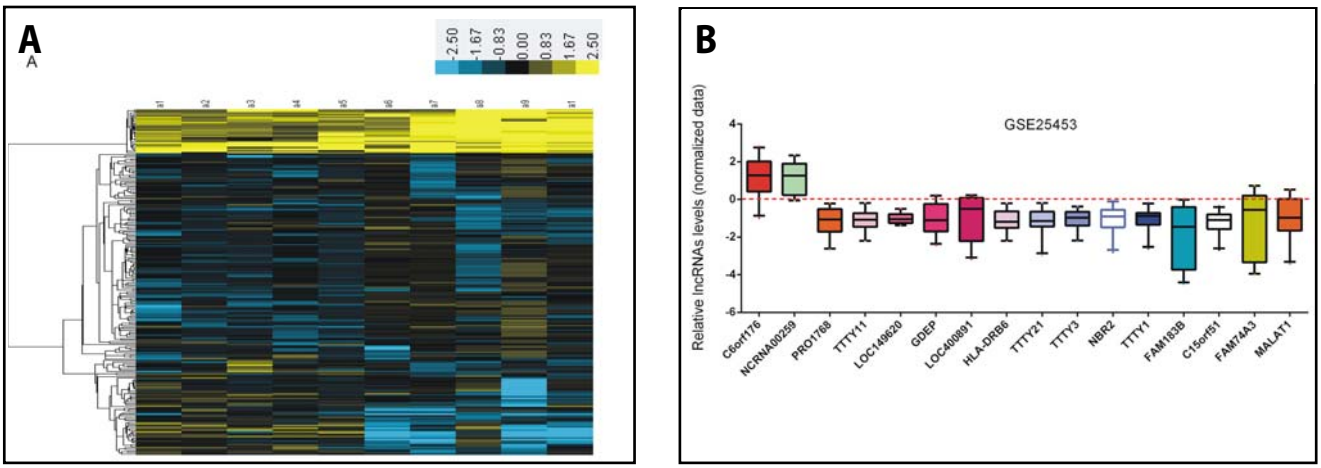

Fig. 1. Expression differences of genes in MTLE. (A) Hierachieal clustering analysis of differentially expressed genes in MTLE. (B) The relative expression levels of 16 DElncs in GSE25453, among which C6orf176 and NCRNA00259 were overexpressed in MTLE, fourteen lncRNAs (PRO1768, TTTY11, LOC149620, GDEP, LOC400891, HLA-DRB6, TTTY21, TTTY3, NBR2, TTTY1, FAM183B, C15orf51, FAM74A3, and MALAT1) were down-regulated in MTLE.

RESULTS

Identification of DEGs and lncRNAs in MTLE

To identify differentially expressed genes (DEGS) in MTLE, DEG identification was performed. A total of 415 DEGs, including 103 up- and 312 down-regulated DEGs, were obtained by using GSE25453. Hierarchical clustering analysis of the DEGs in MTLE and control samples is shown in Figure 1A.

Meanwhile, 16 lncRNAs were identified as differentially expressed lncRNAs. We found C6orf176 and NCRNA00259 were overexpressed in MTLE. However, fourteen lncRNAs (PRO1768, TTTY11, LOC149620, GDEP, LOC400891, HLA-DRB6, TTTY21, TTTY3, NBR2, TTTY1, FAM183B, C15orf51, FAM74A3, and MALAT1) were down-regulated in MTLE (Figure 1B).

Construction of differently expressed lncRNAs related co-expression network in MTLE

Furthermore, we constructed differently expressed lncRNAs related co-expression network to understand roles and functional mechanisms of lncRNAs in MTLE. Pearson's correlation coefficient (PCC) was calculated between the expression levels of each lncRNA-DEG pair in MTLE samples. The co-expressed lncRNA-mRNA pairs with $P<0.01$ were selected in this study. As shown in Figure 2, the dysregulated lncRNA associated network included 16 lncRNAs, 154 differentially expressed genes and and 405 edges.

GO and KEGG Analysis of differentially expressed lncRNAs

Furthermore, we performed bioinformatics analysis for differentially expressed lncRNAs by using their co-expressed mRNAs. GO analysis showed that the dysregulated lncRNAs were associated with positive regulation of transcription from RNA polymerase II promoter, negative regulation of transcription from RNA polymerase II promoter, inflammatory response, extracellular matrix organization, platelet degranulation, retinal ganglion cell axon guidance, metal ion binding, DNA binding transcription factor activity, DNA binding, sequence-specific DNA binding, and calcium ion binding. (Figure 3A-C)

KEGG pathway analysis revealed that dysregulated lncRNAs were primarily enriched in cytokine-cytokine receptor interaction, Ecm receptor Interaction, Pyrimidine metabolism, Focal Adhesion, Jak Stat Signaling Pathway, Mapk Signaling Pathway, Chemokine Signaling Pathway, and Regulation of Actin Cytoskeleton. (Figure 3D)

Functional annotation of key DElncs

In this network, we identified several key lncRNAs, including FAM183B, LOC400891, FAM74A3, LOC149620, PRO1768, TTTY3, NBR2, which were co-expressed with more than 30 mRNA. Furthermore, we performed functional annotation of key DElncs by constructing lncRNA-mRNA-biological processes. We found that NBR2 was mainly involved in regulating transcription. TTTY3, FAM74A3, FAM183B and LOC400891 were associated with regulation of transcription, extracellular matrix organization, and inflammatory response. Meanwhile, we also observed PRO1768 and LOC149620 were involved in regulating extracellular matrix organization, retinal ganglion cell axon guidance, inflammatory response, visceral motor neuron differentiation, and regulation of transcription. (Figure 4)

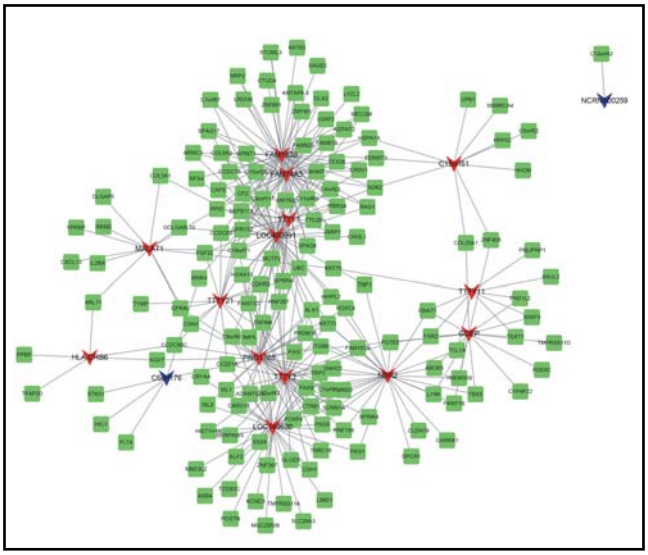

Fig. 2. Co-expression pattern of lncRNAs-mRNAs in MTLE. The interaction network of lncRNAs-mRNAs. The arrow-headed nodes indicate lncRNAs, the squares indicate mRNAs.

DISCUSSION

In recent years, the important roles of lncRNAs in regulating human diseases progression had been implicated. In epilepsy, several groups had paid attention to dysregulated lncRNAs. LncRNA promoters were also found to be mostly hyper-methylated(Xiao *et al.* 2017). Lee *et al.* observed lncRNAs were widely dysregulated in mouse models of localization-related epilepsy by using microarray(Lee *et al.* 2015). Medial temporal lobe epilepsy (MTLE) is the most frequent type of epilepsy(Tatum, 2012). However, whether and how lncRNAs regulating MTLE remained largely unknown. Of note, reannotating data of gene expression microarray provided a novel method to explore special lncRNAs expression pattern in human diseases. For example, Zhang *et al.* identified 1970 lncRNAs in glioma by applying reannotating of gene expression microarray(Zhang *et al.* 2012). In this study, we found 103 up- and 312 down-regulated DEGs by using GSE25453. Meanwhile, 16 lncRNAs (C6orf176, NCRNA00259, PRO1768, TTTY11, LOC149620, GDEP, LOC400891, HLA-DRB6, TTTY21, TTTY3, NBR2, TTTY1, FAM183B, C15orf51, FAM74A3, and MALAT1) were dysregulated in MTLE.

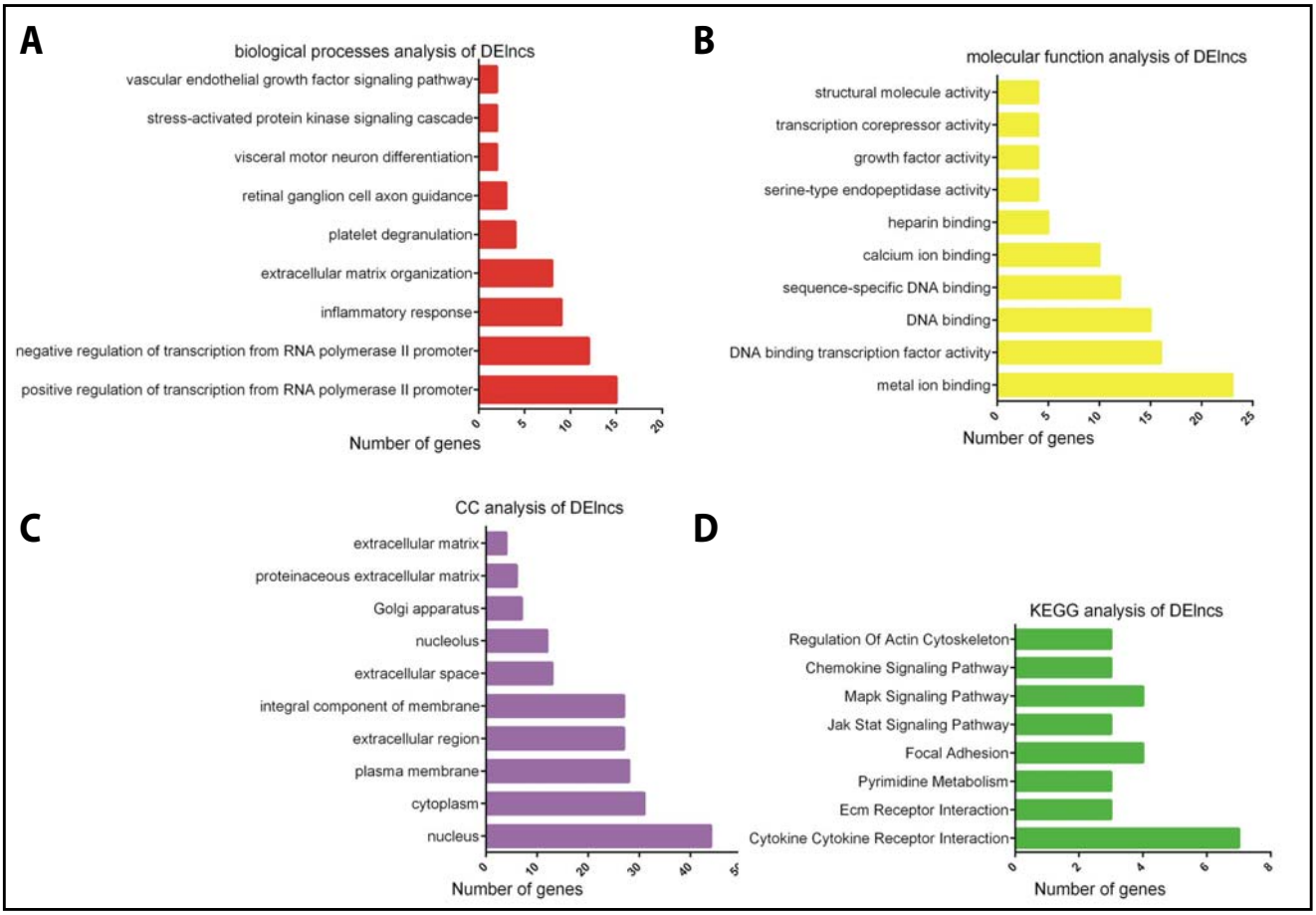

Fig. 3. Functional analysis for the differentially expressed lncRNAs. (A-C) GO analysis of the differentially expressed lncRNAs in biological processes (A), molecular function analysis (B) and Cellular Component (C). (D) KEGG pathway analysis of the differentially expressed lncRNAs.

Previous studies had showed lncRNAs played multiple roles in human diseases by regulating down-stream genes through transcriptional, post-transcriptional, post-translational or epigenetic levels. For instance, lncRNA HOTAIR can recruit PRC2 on hundreds of genes genome-wide(ChuQuinn & Chang, 2012). LncRNA-PAGBC acts as a microRNA sponge and promotes gallbladder tumorigenesis(Wu *et al.* 2017). However, little is known about overall lncRNAs function in MTLE. Co-expression analysis was a useful tool and widely used to classify the putative functions of unknown lncRNAs in different types of human diseases, such as colorectal cancer and venous congestion. In this study, we constructed differently expressed lncRNAs related co-expression network by calculating the Pearson's correlation coefficient value between lncRNA-DEG pair in MTLE samples. A total of 16 lncRNAs, 154 differentially expressed genes and 405 edges were included in MTLE. Several lncRNAs were identified as key lncRNAs by co-expressing with more than 30 mRNA, including FAM183B, LOC400891, FAM74A3, LOC149620, PRO1768, TTTY3, and NBR2.

We also performed bioinformatics analysis for differentially expressed lncRNAs depend on co-expression network. GO analysis showed that the dysregulated lncRNAs were associated with regulation of transcription, inflammatory response, extracellular matrix organization, platelet degranulation, retinal ganglion cell axon guidance, metal ion binding, DNA binding and calcium ion binding. KEGG pathway analysis revealed that dysregulated lncRNAs were primarily enriched in cytokine-cytokine receptor interaction, Ecm receptor Interaction, Pyrimidine metabolism, Focal Adhesion, Jak Stat Signaling Pathway, Mapk Signaling Pathway, Chemokine Signaling Pathway, and Regulation of Actin Cytoskeleton. Our work also evaluated the roles of key lncRNAs in MTLE progression. We found that NBR2 was mainly involved in regulating transcription. TTTY3, FAM74A3, FAM183B and LOC400891 were associated with regulation of transcription, extracellular matrix organization, and inflammatory response. Meanwhile, we also observed PRO1768 and LOC149620 were involved in regulating extracellular matrix organization, retinal ganglion cell axon guidance, inflammatory response, visceral motor neuron differentiation, and regulation of transcription. To the best of our knowledge, this is the first study to report their potential roles in human diseases, special in MTLE.

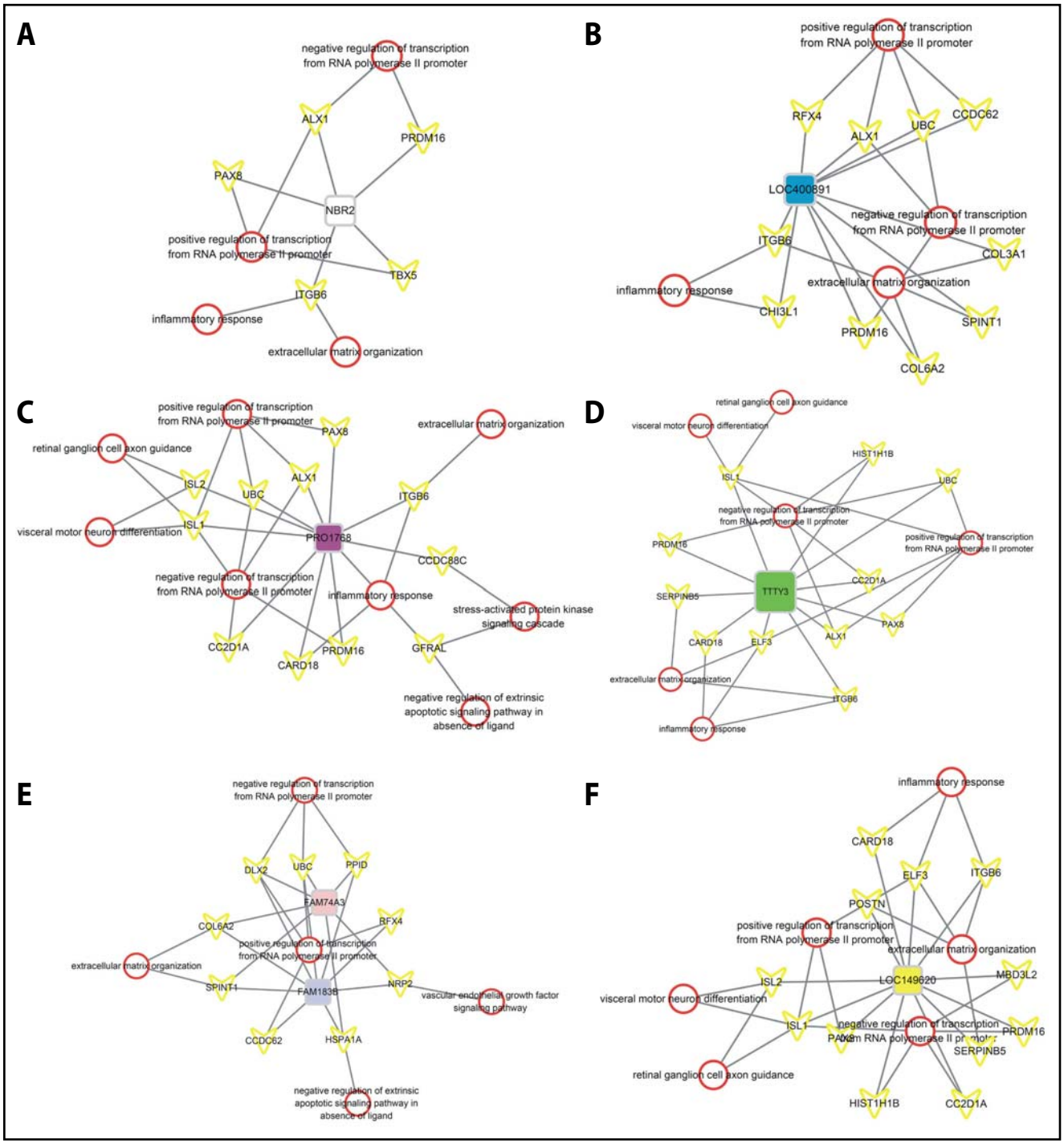

Fig. 4. Signal pathway networks analysis for key lncRNA-mRNA relationships. The squares indicate lncRNAs, the arrow-headed nodes indicate mRNAs and the circles indicate pathways..

CONCLUSION

In conclusion, we analyzed a public dataset GSE25453 to identify differentially expressed lncRNAs and mRNAs in the MTLE. LncRNA co-expression network analysis showed these DElncs were mainly enriched in regulating transcription, inflammatory response, DNA binding, Jak Stat Signaling Pathway, and Mapk Signaling Pathway. Several lncRNAs, including FAM183B, LOC400891, FAM74A3, LOC149620, PRO1768, TTTY3, and NBR2 were identified as key lncRNAs. Meanwhile, lncRNA-mRNA-bp networks were also performed to evaluate their potential roles in MTLE. This study will be useful to explore the potential candidate biomarkers for diagnosis, prognosis, and drug targets of MTLE.

ACKNOWLEDGMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare no financial conflicts of interest.

REFERENCES

1 Avansini SH, de Sousa LB, Secolin, R., Santos ML, Coan AC, & Vieira AS, et al. (2017). MicroRNA hsa-miR-134 is a circulating biomarker for mesial temporal lobe epilepsy. PLoS One. **12**(4): e173060.
2 Bell GS, Neligan A & Sander JW (2014). An unknown quantity--the worldwide prevalence of epilepsy. Epilepsia, **55**(7): 958–962.
3 Chen H, Xu G, Du H, Yi M & Li C (2016). Integrative analysis of gene expression associated with epilepsy in human epilepsy and animal models. Mol Med Rep. **13**(6): 4920–4926.
4 Chu C, Quinn J & Chang HY (2012). Chromatin isolation by RNA purification (ChIRP). J Vis Exp. (61).
5 Decourt B & Sabbagh MN (2011). BACE1 as a potential biomarker for Alzheimer's disease. J Alzheimers Dis. **24** (Suppl 2): 53–59.
6 Gascoigne DK, Cheetham SW, Cattenoz PB, Clark MB, Amaral PP & Taft RJ, et al. (2012). Pinstripe: a suite of programs for integrating transcriptomic and proteomic datasets identifies novel proteins and improves differentiation of protein-coding and non-coding genes. Bioinformatics. **28**(23): 3042–3050.
7 Lee DY, Moon J, Lee ST, Jung KH, Park DK & Yoo JS, et al. (2015). Dysregulation of long non-coding RNAs in mouse models of localization-related epilepsy. Biochem Biophys Res Commun. **462**(4): 433–440.
8 Riva P, Ratti A & Venturin M. (2016). The Long Non-Coding RNAs in Neurodegenerative Diseases: Novel Mechanisms of Pathogenesis. Curr Alzheimer Res. **13**(11): 1219–1231.
9 Shi X, Sun M, Liu H, Yao Y, & Song, Y. (2013). Long non-coding RNAs: a new frontier in the study of human diseases. Cancer Lett, **339**(2): 159–166.
10 Tatum WT (2012). Mesial temporal lobe epilepsy. J Clin Neurophysiol, **29**(5): 356–365.
11 Venugopal AK, Sameer KG, Mahadevan A., Selvan LD, Marimuthu, A, & Dikshit JB, et al. (2012). Transcriptomic Profiling of Medial Temporal Lobe Epilepsy. J Proteomics Bioinform. **5**(2).
12 Wan X, Huang W, Yang S, Zhang Y, Pu H, & Fu F, et al. (2016). Identification of androgen-responsive lncRNAs as diagnostic and prognostic markers for prostate cancer. Oncotarget. **7**(37): 60503–60518.
13 Wu XS, Wang F, Li HF, Hu YP, Jiang L, & Zhang, F., et al. (2017). LncRNA-PAGBC acts as a microRNA sponge and promotes gallbladder tumorigenesis. EMBO Rep. **18**(10): 1837–1853.
14 Xiao W, Cao Y, Long H, Luo Z, Li S & Deng N, et al. (2017). Genome-Wide DNA Methylation Patterns Analysis of Noncoding RNAs in Temporal Lobe Epilepsy Patients. Mol Neurobiol.
15 Zhang X, Sun S, Pu JK, Tsang AC, Lee D & Man VO, et al. (2012). Long non-coding RNA expression profiles predict clinical phenotypes in glioma. Neurobiol Dis. **48**(1): 1–8.