MP84-10

The expression profiling and possible regulating targets of Long Non-Coding RNA in rats with cavernous nerve injury erectile dysfunction

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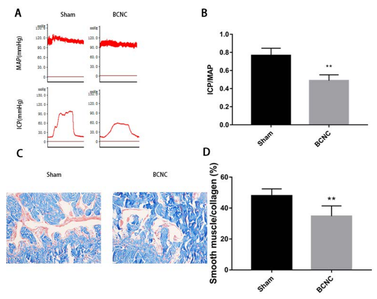
Introduction

Long Non-Coding RNA (LncRNA) was discovered to be associated with erectile dysfunction in diabetes and aging. Whereas, lncRNA expression profile in cavernous nerve injury erectile dysfunction (CNI-ED) is unknown. Hence, the aim of our study is to detect abnormal lncRNA expression in CNI-ED and analyze possible target genes and pathways.

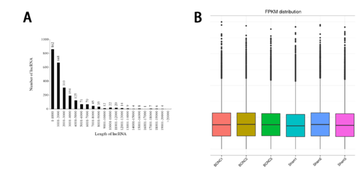
Method

Twenty-two Sprague Dawley rats were divided into bilateral cavernous nerve crush (BCNC) and sham groups. Four weeks after surgery, erectile function and histological change in the corpus cavernosum were evaluated. Total RNA from 3 rats from each group was isolated and processed to analyze the lncRNA, miRNA and mRNA expression profiling by transcriptome sequencing. The 17 dysregulated lncRNA profiles were chosen directly and further validated in another 8 rats per each group by quantitative real-time polymerase chain (PCR) reaction. The enrichment analysis of gene ontology-term analysis and Kyoto Encyclopedia of Genes and Genomes were performed by DAVID database. The overlapped predicted miRNAs as downstream targets of three validated lncRNAs via qRT-PCR and upstream regulators of differentially expression mRNAs were identified and final LncRNA-miRNA-mRNA network was constructed.

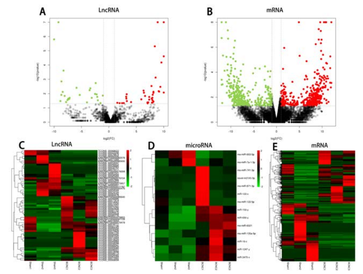
Results



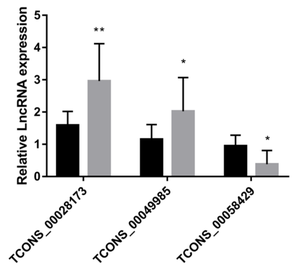
(A) Representative ICP and MAP recording of the BCNC and sham groups. (B) The cavernous electrostimulation results in the two groups are expressed as ICP/MAP. (C) Detection of fibrosis in the BCNC and Sham groups by Masson's trichrome staining. (D) Bar graph of smooth muscle/collagen. Bar graphs represent means ± standard deviation. *p<0.05 and **p<0.01 compared with the Sham group.



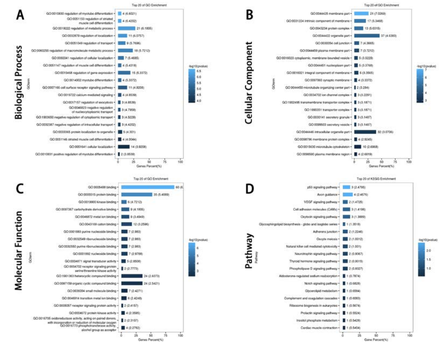
(A) Length of lncRNAs identified by RNA-seq. (B) Box plots of FPKM value of lncRNAs in six samples. (n=3 for each group)



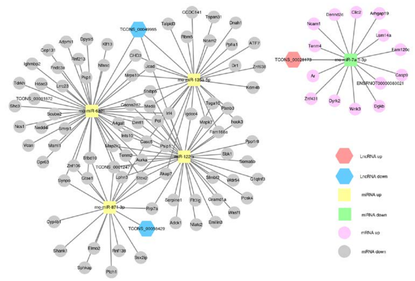
(A) Volcano plots visualizing the differential expression of lncRNAs. The vertical lines correspond to 2.0-fold up and down, and the horizontal one represents a p-value of 0.05. (B) Volcano plots visualizing the differential expression of mRNAs. (C) Hierarchical cluster analysis (heat map) of RNA-seq data was used to assess the significant expression of lncRNAs when comparing the BCNC and Sham groups. (D) Hierarchical cluster analysis (heat map) of RNA-seq data was used to assess the significant expression of miRNAs when comparing the BCNC and Sham groups. (E) Hierarchical cluster analysis (heat map) of RNA-seq data was used to assess the significant expression of mRNAs when comparing the BCNC and Sham groups.



Differential expression of lncRNAs between the BCNC and Sham groups by quantitative PCR. The bar graphs represent means ± standard deviation. *p<0.05 and **p<0.01 compared with the Sham group.



GO and pathway analysis of dysregulated mRNAs in the lncRNA–miRNA–mRNA network. Top 20 enrichment terms of the (A) biological processes, (B) cellular component, and (C) molecular function; (D) KEGG pathways enrichment for the mRNAs in the lncRNA–miRNA–mRNA network.



View of the lncRNA–miRNA–mRNA network. The red and blue hexagons stand for the up-regulated and down-regulated lncRNAs, respectively. The yellow and green squares represent the up-regulated and down-regulated miRNAs, respectively. The pink and grey circles denote the up-regulated and down-regulated mRNAs, respectively.

Primers used for qRT-PCR analysis of lncRNAs

Target ID	Forward Primer	Reverse Primer
ENSRNOT00000077787	F:5′ AGACGCCGAATGACAAA 3′	R:5′ TGCTGAGGCTGCTGGAT 3′
TCONS_00001531	F:5′ AGTAGCTTTGCGGAGTGC 3′	R:5′ TGTTCCCAGCCATCTTCA 3′
TCONS_00005632	F:5′ GCACTGGAAGAAGGGAAAG 3′	R:5′ CCTGACATTCTAAGCCTCTG 3′
TCONS_00014063	F:5′ CGCTAACCCTGCTCCGTCTC 3′	R:5′ CAGCCAGCCTGGATCTTCTA 3′
TCONS_00027746	F:5′ GGAGGGCACCAAGGATG 3′	R:5′ CTCGGTCTGATGTTTCACAAA 3′
TCONS_00028173	F:5′ CCACATAAGGACATAAAGAAGCTG 3′	R:5′ GGATGTAGGTGGAGGTTGGA 3′

Target ID	Forward Primer	Reverse Primer
TCONS_00034544	F:5′ GTGGTCATACGCAACGC 3′	R:5′ AGCACCCAGTCCCAGTA 3′
TCONS_00049985	F:5′ GGCTTGGCTTGGATTGG 3′	R:5′ GCCCTGGTTGTATGCTATT 3′
TCONS_00049986	F:5′ AAGGCCTGAAACGTTGACTG 3′	R:5′ TGTGTGTGTGTGAGGGTGAA 3′
TCONS_00058429	F:5′ CCCGAGCAAATGGGTAAA 3′	R:5′ GGGCACGCTGTGAGGAA 3′
TCONS_00063042	F:5′ GGAAGGGAGGCAGAAGG 3′	R:5′ GCAGTGCTCATAGGTGGG 3′
TCONS_00063284	F:5′ GTCGTAGATTTCAGCGTAT 3′	R:5′ AGCTCTTTGGCACTTTT 3′

Target ID	Forward Primer	Reverse Primer
TCONS_00063405	F:5′ TGTTGTGCTCCAGCTATG 3′	R:5′ GGACTCCCAGAATCCAAG 3′
TCONS_00063406	F:5′ CTGCTTTATTCCAGGACAAGAC 3′	R:5′ CCTGAGCAATGGCAACTCC 3′
TCONS_00069497	F:5′ GCCTTAGGTATTCCTGACA 3′	R:5′ CTCCTCAGTCTCGGTCAT 3′
TCONS_00074998	F:5′ AGTGGCAAACAGGATGA 3′	R:5′ CAGGACAGGGCTTAGTG 3′
TCONS_00075340	F:5′ GTTGTGACTTACGGTAGAGG 3′	R:5′ GATGCCAGCAATCCCTA 3′

Conflict of interest

The authors declare there are no competing interests.

Conclusion

The results indicated significantly altered expression profiles of lncRNAs, miRNA and mRNAs between BCNC and sham group. This study may shed light on the further research on CNI-ED and may be helpful for discovering a new therapeutic target for CNI-ED.