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Study of Mouse Testis-specific miR-471 expression and function

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Abstracts ***************

MiRNA plays important regulatory roles in various normal and malignant biological processes, however, its function in male germ cell development remains poorly elucidated. In this study we explored temporal expression pattern of mouse testis-specific miR-471 in mouse testes. Furthermore, we investigated the function of miR-471 using established mouse spermatogonial stem cell in vitro transgenic model. We discovered that 3T3 stable cell line overexpressing miR-471 proliferate more actively than control cell line. Similarly, overexpressing miR-471 in cultured mouse spermatogonial stem cells also promotes cell proliferation. These results suggest that miR-471 may trigger cell proliferation during mouse testis development. This work was supported by grants No.90919020, No.10XD1402200, No.2010CB945001 and No.2009ZX08006-010B.

Introduction **********

MiRNA is a family of ~22nt short single-strand RNAs, which exists in various organisms and plays crucial roles in regulating various biological processes. Series of large-scale high-throughput library screening and microarray studies have found and characterized a large reservoir of miRNAs. MiR-471, a mouse testis-specific miRNA, is located wthin a miRNA-rich region in mouse X chromosome. However, the definitive functions of which are poorly understood, especially for those related to male germ cell development.

Results ***************

1 Genomic context of miR-471

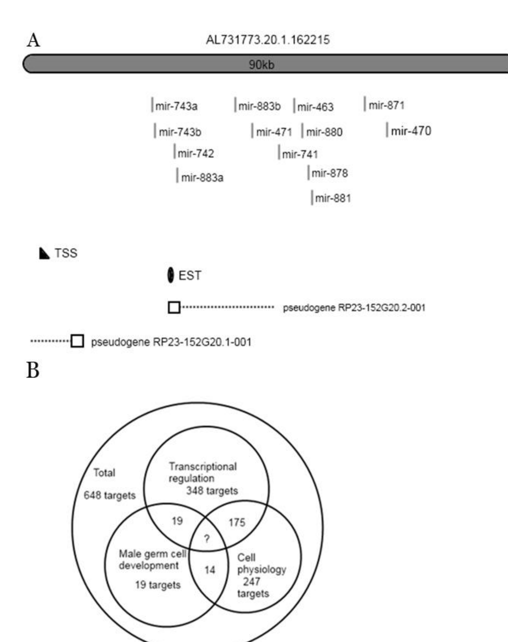

Fig. 1. (A) miR-471 is located wthin a miRNA-rich region in mouse X chromosome. A total of 13 miRNA genes concentrate in ~40kb of genomic region. This locus also contains transcriptional features like transcription start site (TSS), processed pseudogenes with EST data proving transcriptional expression. (B) Target gene prediction of X-linked miRNA cluster. Predicted target gene sets were further clustered depending on previously known functions annotated in GO terms.

2 Temporal expression pattern of miR-471 in mouse testes

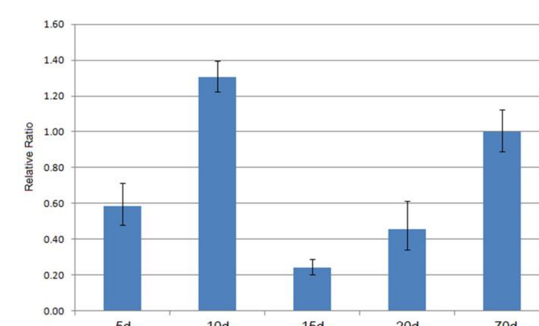

Fig.2.Temporal expression pattern of miR-471 in mouse testes. Expression levels of miR-471 in testes from mice of different ages as revealed by qPCR.

3 PCR cloning and plasmid construct of miR-471

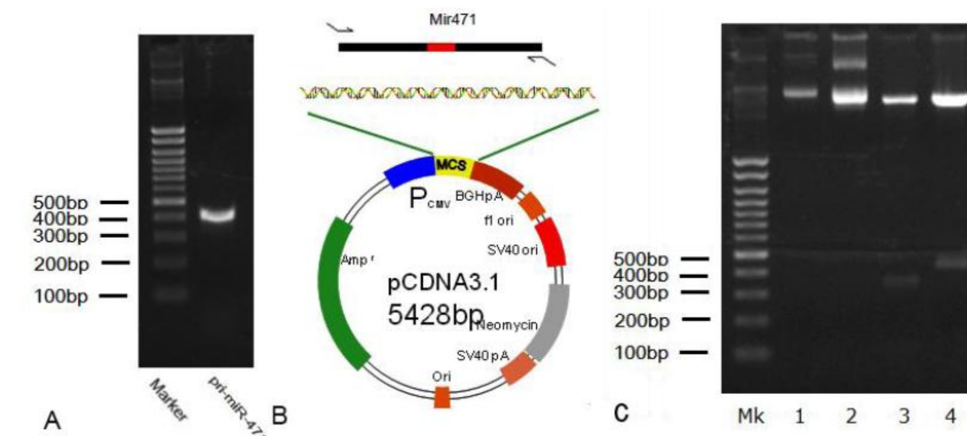

Fig. 3. PCR cloning and plasmid construct of miR-471. (A) PCR amplification of genomic DNA encompassing miR-471 resulted in a specific product band of 425bp; (B) Rationale of miR-471 cloning and expression vector constructing; (C) Restriction enzyme cut of expression construct. Lane 1: pcDNA3.1; lane 2: pcDNA3.1-pri-miR-471; lane 3: pcDNA3.1 EcoRI-XhoI double digestion; lane 4: pcDNA3.1-pri-miR-471 EcoRI-XhoI double digestion.

4 Overexpression of miR-471 in 3T3 cells

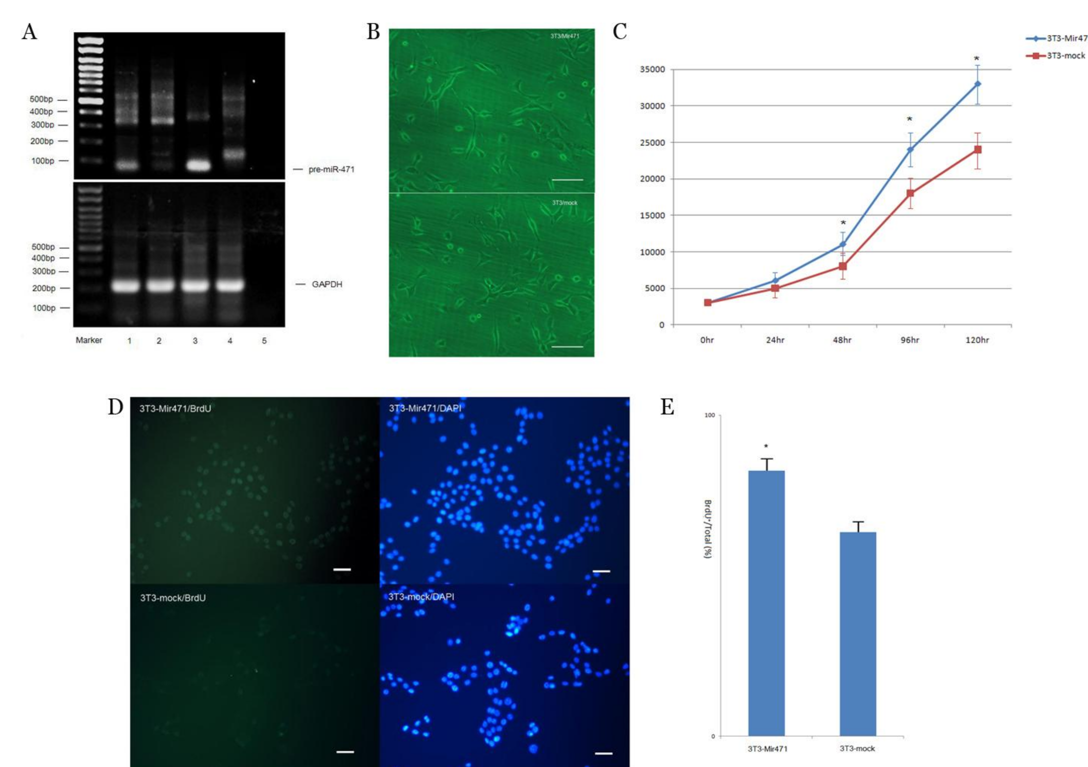

Fig.4.Overexpression of miR-471 in 3T3 cells. (A) RT-PCR detection after transient transfection. Lane 1: 3T3/miR471 24hr after transfection; lane 2: 3T3/mock 24hr after transfection; lane 3: 3T3/miR471 36hr after transfection; lane 4: 3T3/mock 36hr after transfection; lane 5: negative control. (B) No discernible morphological difference is presented between 3T3-miR-471 (upper panel) and 3T3-mock (lower panel) cell lines (bar=100μm). (C) Comparison of growth curves of 3T3-miR-471 and 3T3-mock stable cell lines. Asterisk indicates statistical significance ($p<0.05$). (D) BrdU cell proliferation assay of 3T3-Mir471 and 3T3-mock stable cells. Upper-left and lower-left panels are BrdU immunofluorescent detections of synchronized 3T3-Mir471 and 3T3-mock stable cells, respectively; upper-right and lower-right panels are DAPI stainings (bar=100μm). (E) Ratios of BrdU+ cells in total cell count, which is significantly higher in 3T3-miR-471 than in 3T3-mock ($n=3$, $p<0.05$).

5 Overexpression of miR-471 in mouse SSC cultured in vitro

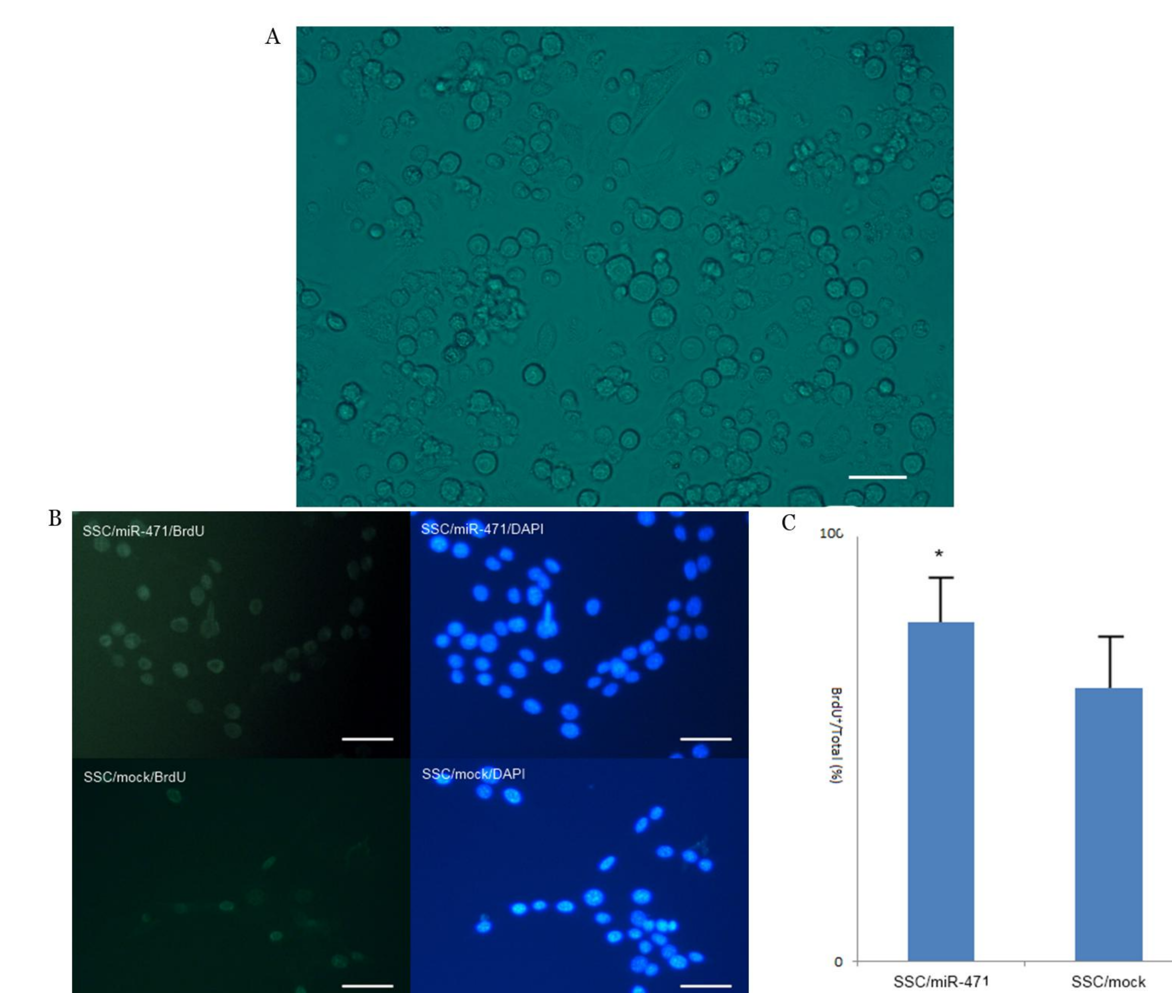

Fig.5.Overexpression of miR-471 in mouse SSC cultured in vitro. (A) Mouse primary SSC culture, 12hr after isolation. (bar=100μm) (B) BrdU cell proliferation assay of SSC/miR-471 (transfected with pcDNA3.1-pri-miR-471) and SSC/mock (transfected with pcDNA3.1-mock) cells. Upper-left and lower-left panels are BrdU immunofluorescent detections of synchronized SSC/miR-471 and SSC/mock cells, respectively; upper-right and lower-right panels are DAPI stainings. (bar=100μm) (C) Ratios of BrdU+ cells in total cell counts, which is significantly higher in SSC/miR-471 than in SSC/mock ($n=3$, $p<0.05$).

Conclusion **************

1.Temporal expression pattern of miR-471 in mouse testis;

2.“Proof-of-principle” overexpression system of miRNA in cell lines and SSCs in vitro, which could be scaled for high-throughput researches or applied in functional studies of other testis-specific miRNAs;

3.MiR-471 may promote cell proliferation during mouse testis development, which is crucial for constant regeneration of male gametes.

References

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