LncRNA Slfn5os Regulates the Survival and Testosterone Production in Tm3 Leydig Cell Lines

Kaimin Guo, Zhanmeng Zhu, Zhiyi Zhao, Xinzheng Zhao, Jiantao Zhao, Lingyun Liu and Hongliang Wang*

Department of Andrology, First Hospital of Jilin University, Changchun, China

Abstract

Long non-coding RNAs (lncRNAs) have been reported to regulate the spermatogenesis. In this study, we aim to characterize the expression pattern and roles of lncRNA schlafen 5, opposite strand (Slfn5os) in the testis of adult

Polymerase Chain Reaction (RT-PCR). The localization of lncRNA Slfn5os and Slfn5 was determined by Fluorescence In situ Hybridization (FISH). TM3 Leydig cell line was used as a cellular model to study the function of Slfn5os. The survival of TM3 cells upon Slfn5os knockdown or overexpression was assessed by Cell Count Kit-8 (CCK-8) viability

of Slfn5os. The expression level of Slfn5os negatively regulates the mRNA level of Slfn5. In addition, forced expression of Slfn5os impaired the survival and testosterone production in TM3 cells, while Slfn5os silencing showed the opposite

survival and testosterone production in Leydig cells.

Keywords: [illegible]

Introduction

[illegible]

***Corresponding authors:**

Received: **Editor assigned:** 28-
Reviewed:
Revised: **Published:**

Citation: Guo K, Zhu Z, Zhao Z, Zhao X, Zhao J, et al. (2022) LncRNA Slfn5os Regulates the Survival and Testosterone Production in Tm3 Leydig Cell Lines. Cell

Slfn5os (schlafen 5, opposite strand, 1700003F12Rik) is a novel lncRNA identified in mouse testis, and it has been found to be regulated by an endocrine disruptor, Di-2-Ethyl Hexyl Phthalate (DEHP) [20]

It is transcribed from the opposite strand of Slfn5 (Schlafen family member 5) gene. However, its tissue-specific expression and functional role in testis is largely unknown. Elucidating its expression pattern and role in testis can provide insights into the novel mechanism of

Material and Methods

Animals and cell culture

[illegible] 57 /6 [illegible] 12- [illegible] /12- [illegible]

[illegible] 3 [illegible] -2. [illegible] 3 [illegible] 1:1 [illegible] : 12 [illegible] 1% [illegible]), 2.5% [illegible] 5% [illegible] 37+ [illegible] 5% [illegible] -2. [illegible] 10% [illegible] 1% [illegible]

Cell transfection

[illegible] 5 [illegible] 5 [illegible] 3.1 [illegible] 3.1- [illegible] 5 [illegible] 3 [illegible] 6- [illegible] 1 10^5 [illegible] 24 [illegible] 2000 [illegible] 3 [illegible] 6- [illegible] 60% [illegible] 50 [illegible] 6 [illegible] 8 [illegible] 48 [illegible]

Fluorescence *In situ* Hybridization (FISH)

[illegible] 3 [illegible] 4° [illegible] 4% [illegible] 5 [illegible] 21 [illegible] (1:500 [illegible] 3 (1:50 [illegible] 1 [illegible] 1:100 [illegible] 1 [illegible] 5 [illegible]

[illegible] 5 :5 - [illegible] -3 -(30 [illegible])

[illegible] 5: 5 - [illegible] -3 -(25 [illegible])

Quantitative RT-PCR and RT-PCR (reverse-transcription PCR)

[illegible] (2 [illegible] 7500 [illegible] : 95° 2 [illegible] 40 [illegible] 95° 30 [illegible] 60° 30 [illegible] 72° 60 [illegible] 2 [illegible]

[illegible] 5 [illegible] 5 [illegible] 42 [illegible] 94+ [illegible] 45 [illegible] 57° [illegible] 1 [illegible] 72° [illegible] 90 [illegible] 1% [illegible] 1, [illegible] 5 [illegible] 5 [illegible] 5 [illegible] 45 [illegible]

Cell proliferation assays

[illegible] 8 [illegible] 8; [illegible] 96 [illegible] 5000 [illegible] 48 [illegible] 10 [illegible] 8 [illegible] 1 [illegible] 37° [illegible] 10 [illegible] 490 [illegible]

Apoptosis detection by [illegible]ow cytometry

[illegible] 3 [illegible] (4 [illegible] 1 106 [illegible] 5 [illegible] 5 [illegible] 30 [illegible]

Table 1: Primer sequences.

Gene	Primer Sequence
Slfn5 (RT-PCR)	
Slfn5os (RT-PCR)	
Slfn5 (qPCR)	
Star (qPCR)	
Cyp11a (qPCR)	
Lhr (qPCR)	
Hsd3b1 (qPCR)	

[illegible] 400 [illegible] ([illegible]).

Testosterone measurement by ELISA (Enzyme-Linked Immuno Sorbent Assay)

[illegible] 25 [illegible] 100 [illegible] 1 [illegible] 1 [illegible] 15 [illegible] 450 [illegible]

Citation:

[illegible]

Slfn5os knockdown improves TM3 cell proliferation and testosterone secretion

[illegible]

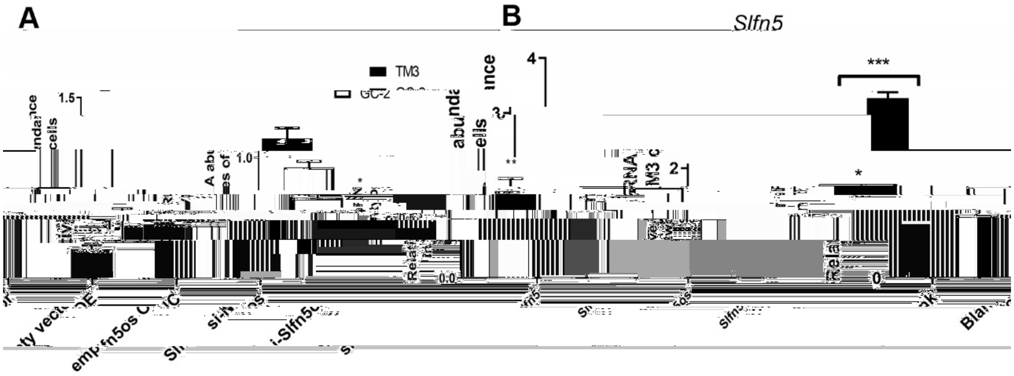

Figure 2: The impact of Slfn5os on Slfn5 expression. (A) The expression levels of Slfn5os on Slfn5were examined by RT-qPCR in TM3 cells (mouse Leydig cell line) and GC-2 cell line (mouse spermatocytes). (B) mRNA expression of Slfn5 in TM3 cells after the transfection of Slfn5os empty vector, Slfn5os expression plasmid, si-NC or si-

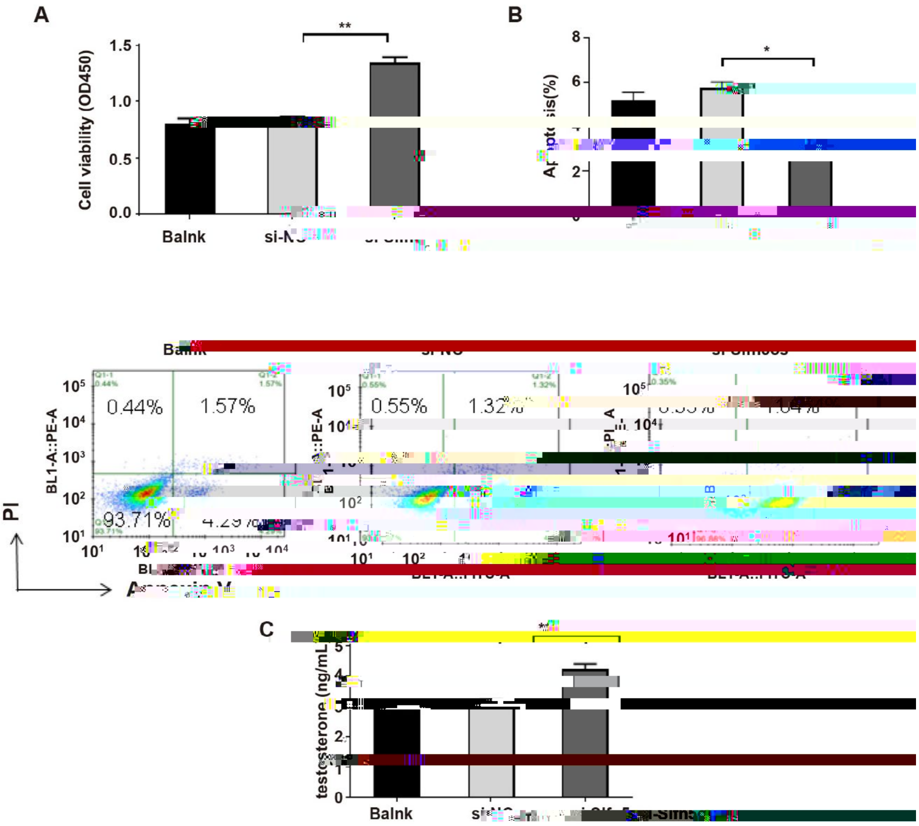

Figure 3: Slfn5os knockdown promotes TM3 cell proliferation, inhibits cell apoptosis and increases testosterone secretion. (A) CCK-8 proliferation assay in

[illegible]

Slfn5os overexpression impairs cell proliferation and testosterone secretion

[illegible]

Slfn5os modulates the expression of steroidogenic genes and enzymes in TM3 Leydig cells

[illegible]

[illegible]

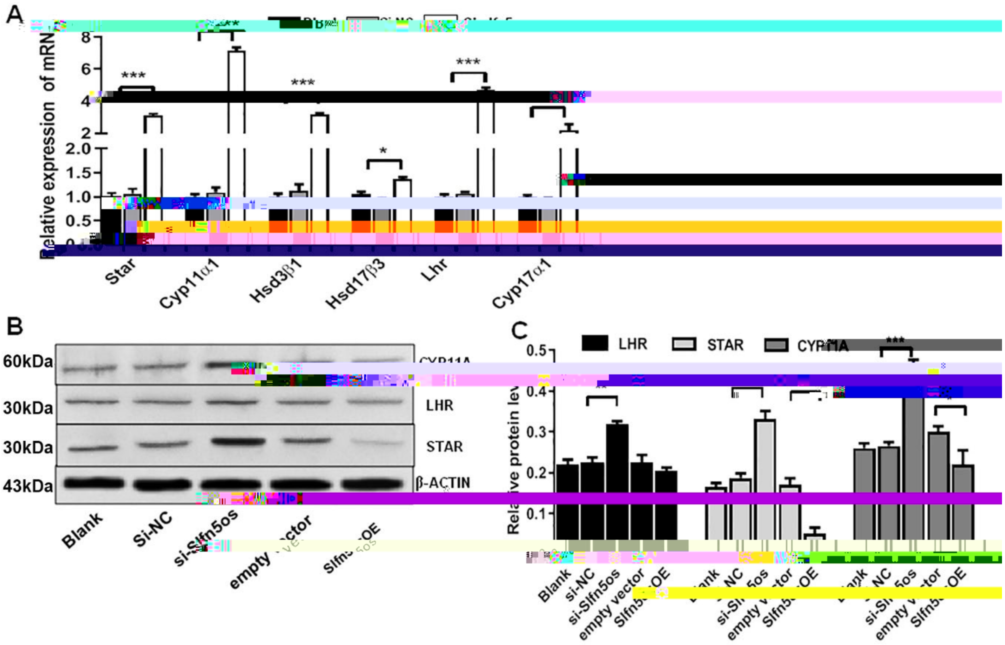

Figure 5: Slfn5os knockdown promotes the expression of steroidogenic genes in TM3 cells. (A) RT-qPCR analysis of the expression of key steroidogenic genes in TM3

[illegible]

[illegible]

[illegible]

[illegible]

Conclusion

[illegible]

Declarations

Ethics Approval and Consent to Participate

[illegible]

Consent for Publication

[illegible]

Availability of Data and Material

[illegible]

Competing Interest

[illegible]

Funding

[illegible] ([illegible].81901534) [illegible] ([illegible].3 5214506428).

Author’s Contributions

[illegible]

Acknowledgement

[illegible]

References

1. Spermatogenesis
2. Regulation of mammalian spermatogenesis by miRNAs.
3. Yokonishi T, McKey J, Ide S, Capel B (2020) Sertoli cell ablation and replacement of the spermatogonial niche in mouse
5. Zhan X, Zhang J, Li S, Zhang X, Li L, et al. (2020) Monocyte Chemoattractant during regeneration

Neuregulin 1 Regulates Proliferation of Leydig Cells to Support Spermatogenesis and Sexual Behavior in Adult Mice

8. Kaprara A, Huhtaniemi IT (2018) of mice and men

Androgen Actions in the Testis and the Regulation of Spermatogenesis

10. Novel Gene Regulation in Normal and Abnormal Spermatogenesis
11. The Presence *In vitro* 2325.
12. Cheuquemán C, Maldonado R (2021) epigenetic factors from spermatogenesis to transgenerational inheritance. Biol
13. The Role of Non-coding RNAs in Oncology.

The roles of microRNAs in regulation of mammalian spermatogenesis

15. Production

Long non-coding RNAs (lncRNAs) in spermatogenesis and male infertility

Long noncoding . J Anim Sci

18. Song X, Kyi-Tha-Thu C, Takizawa T, Naing BT, Takizawa T (2018) coding RNAs

Otsuka K, Matsubara S, Shiraishi A, Takei N, Satoh Yubar5100035[illegible]