Exosome-Derived microRNAs in Sertoli Cells Inhibit Spermatogonial Apoptosis

Huihui Gao

Northwest A&F University: Northwest Agriculture and Forestry University

Heran Cao

Northwest A&F University: Northwest Agriculture and Forestry University

Tianqi Jin

Northwest A&F University: Northwest Agriculture and Forestry University

Guofan Peng

Northwest A&F University: Northwest Agriculture and Forestry University

Yining Chen

Northwest A&F University: Northwest Agriculture and Forestry University

Wenxian Zeng

Northwest A&F University: Northwest Agriculture and Forestry University

Chao Zhu

Northwest A&F University: Northwest Agriculture and Forestry University

Jian Du

Tsinghua University

WuZi Dong (✉ dongwuzi@nwafu.edu.cn)

Northwest A&F University: Northwest Agriculture and Forestry University

Research

Keywords: Sertoli cells, exosomes, miRNAs, spermatogonial, apoptosis

Posted Date: March 31st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-352948/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Spermatogenesis is a highly complicated biological process that occurs in the epithelium of the seminiferous tubules. It is regulated by a complex network of endocrine and paracrine factors and juxtacrine testicular cross-talk . Sertoli cells (SCs) play a key role in spermatogenesis due to their production of trophic, differentiation and immune-modulating factors. However, many of the molecular pathways of SCs action remain controversial and unclear. Recently, research has focused on exosomes as an important mechanism of intercellular communication.

Results

We found that the exosomes derived from SCs (SC-Exos) significantly inhibited the apoptosis of the primary spermatogonial stem cells (SSCs). Total of 1016 miRNAs in SCs and 556 miRNAs in SC-Exos were detected using microRNA (miRNA) high-throughput sequencing. Further, 294 miRNAs were differentially expressed between SCs and SC-Exos. Based on the GO and KEGG analyses, the target genes of 37 (high-expressed in exosomes and RPM>1000) selected miRNAs were involved in multiple biological pathways, including the MAPK signaling pathway and PI3K/AKT signaling pathway. And miR-10b is one of the top ten exosomes with relatively large enrichment of microRNA. In addition, the overexpression of miR-10b down-regulated expression of the target KLF4 to reduce spermatogonial apoptosis in SSCs or C18-4 cell line.

Conclusions

The study indicated a large number of small RNAs loaded in exosomes was secreted from the donor SCs to target spermatogonial regulated the apoptosis. And miR-10b inhibits the apoptosis of spermatogonia through the target gene KLF4.

Full Text

This preprint is available for [download as a PDF](#).

Tables

Table1 Summary of small RNA sequencing data in Sertoli cells and exosomes libraries

Type	Sertoli cells		Exosomes	
	Reads	Percent (%)	Reads	Percent (%)
Total reads	14,271,751	100	15,549,820	100
3'adapter null	631,371	4.42	617,486	3.97
Insert null	22,984	0.16	3,436	0.02
5'adapter contaminats	10,376	0.07	1,028	0.01
Removed low quality	176,013	1.23	441,515	2.84
Smaller than 17nt	384,758	2.70	138,161	0.89
Clean reads	13,046,249	91.41	14,348,194	92.27

Table 2 Unique miRNAs expressed in Sertoli cells and exosomes

Sertoli cells						Exosomes		
miRNA ID	Reads	RPM	miRNA ID	Reads	RPM	miRNA ID	Reads	RPM
mmu-miR-135b-5p	903	81.5691	mmu-miR-135b-3p	82	7.4072	mmu-miR-6538	5	4.4586
mmu-miR-3068-5p	903	81.5691	mmu-miR-1193-3p	80	7.2265			
mmu-miR-455-3p	767	69.284	mmu-miR-345-5p	79	7.1362			
mmu-miR-221-5p	673	60.7929	mmu-miR-324-3p	76	6.8652			
mmu-miR-152-5p	620	56.0053	mmu-miR-369-5p	74	6.6845			
mmu-miR-199b-5p	439	39.6554	mmu-miR-669l-5p	74	6.6845			
mmu-miR-30b-3p	415	37.4874	mmu-miR-34b-5p	71	6.4135			
mmu-miR-669a-5p	317	28.635	mmu-miR-466b-3p	71	6.4135			
mmu-miR-669p-5p	317	28.635	mmu-miR-466c-3p	71	6.4135			
mmu-miR-301a-5p	248	22.4021	mmu-miR-466p-3p	71	6.4135			
mmu-miR-411-3p	243	21.9505	mmu-miR-7686-5p	71	6.4135			
mmu-miR-30c-1-3p	227	20.5052	mmu-miR-376c-3p	70	6.3232			
mmu-miR-24-1-5p	170	15.3563	mmu-miR-704	69	6.2329			
mmu-miR-467c-5p	168	15.1756	mmu-miR-130a-5p	64	5.7812			
mmu-miR-99a-3p	140	12.6464	mmu-miR-669d-5p	63	5.6909			
mmu-miR-26a-2-3p	123	11.1107	mmu-miR-299a-5p	62	5.6005			
mmu-miR-379-3p	122	11.0204	mmu-miR-324-5p	57	5.1489			
mmu-miR-	122	11.0204	mmu-miR-	57	5.1489			

5100			467d-5p		
mmu-miR-29b-3p	120	10.8397	mmu-miR-708-5p	55	4.9682
mmu-miR-17-3p	120	10.8397	mmu-miR-497a-5p	54	4.8779
mmu-miR-148b-5p	117	10.5687	mmu-miR-466c-5p	53	4.7876
mmu-miR-700-5p	99	8.9428	mmu-miR-669b-5p	52	4.6972
mmu-miR-7226-3p	96	8.6718	mmu-miR-301a-3p	47	4.2456
mmu-miR-669o-5p	93	8.4008	mmu-miR-1948-5p	47	4.2456
mmu-miR-1843a-3p	92	8.3105	mmu-miR-467a-3p	45	4.0649
mmu-miR-6516-5p	90	8.1298	mmu-miR-669e-5p	45	4.0649
mmu-miR-18a-5p	84	7.5878			

The expression is presented as reads per million (RPM) with Mean RPM >4.

Figures

Figure 1

Separation and purification of primary Sertoli cells (A) The morphology of cultured primary SCs and the proliferation curve of SCs in DMEM/F12 supplemented with 10% (v/v) FBS. Verified the cell purity by PCR(B), immunocytochemical (C), C: Sertoli cells, T: testes, M: marker. Data are presented as Mean $\pm$ Standard deviation. Scale bar, 100 μ m.

Figure 2

SC-exosomes characterization TEM (A) and SEM(B) images of SC-exosomes showing a homogeneous population of exosomes; (C) Western blot analysis of common exosome markers, where 20 μ g of total protein was loaded in each lane; (D) Hydrodynamic size distribution of SC-exosomes measured by nanosight. Scale bar, 100 nm.

Figure 3

SC-exosomes target spermatogonia to inhibit apoptosis. (A) Dil-exosomes incubation spermatogonia after 12h, the positive signal was detected. Control: Dil incubation spermatogonia. (B) The viability of spermatogonia after exosome processing. (C) Annexin V/FITC staining of apoptotic cells after exosomes treatment. Data are presented as Mean $\pm$ Standard deviation. ** $p < 0.01$; Scale bar, 100 μ m.

Figure 4

Profiling of small RNAs in mouse Sertoli cells and exosomes samples. (A) Correlation analysis of the small RNAs in SCs and exosomes; (B) Venn diagram showing the profile of kinds of small RNAs expressed in SCs and exosomes; (C) Size and frequency distribution of detected small RNAs (19-40nt); (D) The relative abundance of different classes of small RNAs.

Figure 5

Profiling of miRNAs in mouse Sertoli cells and exosomes samples. (A) Venn diagram showing the profile of miRNAs expressed in SCs and exosomes; (B) Volcano plot generated by clustering of the variable miRNAs in SCs and exosomes; (C-D) The pie chart of ten most abundant miRNAs. C: Sertoli cells. D: Exosomes. MiRNAs differed in two samples were colored and marked.

Figure 6

Gene ontology and KEGG analysis of the predicted targets for high expressed miRNAs in exosomes. The targeted genes of high expressed miRNAs (A) is analyzed in this figure. The right-hand-side scale is the targeted gene numbers corresponding to the GO terms. The left-hand-side scale is the $-\log_{10}$ (P-Value) of GO terms. Ten GO terms for each process are listed. (B) KEGG analysis of the 30 most enriched pathways. The right-hand-side scale is the targeted gene numbers corresponding to the KEGG pathway. The left-hand-side scale is the $-\log_{10}$ (P-Value) of KEGG pathway.

Figure 7

Expression of selected different expression miRNAs between Sertoli cells and exosomes detected by RT-qPCR and miRNA-seq. (A) Expression of miR-26a-5p; (B) Expression of let-7i-5p; (C) Expression of miR-148a-3p; (D) Expression of miR-10b-5p; Small RNAs expression from RT-qPCR represented by lines on the top and values are shown on the left vertical axis as relative abundance. Small RNA expression from miRNA-seq represented by bars on the bottom and values are shown on the right vertical axis as RPM (normalized reads number). Data are presented as Mean $\pm$ Standard deviation. *, $p < 0.05$, ** $p < 0.01$.

Figure 8

miR-10b inhibits spermatogonia inhibit by targeting KLF4. (A) The expression of miR-10b after transfected with mimics (B) CCK-8 was used to detect the effect of miR-10b on spermatogonia. (C) The statistical results and representative figures of the effect of miR-10b on the apoptosis of spermatogonia as detected by TUNEL. (D) Western blot showing the apoptotic markers after miR-10b treatment. (E-F) The expression KLF4 detected by RT-PCR and Western blot. Data are presented as Mean $\pm$ Standard deviation. ** $p < 0.01$; Scale bar, 100 μm .

Figure 9

Exosome-derived miR-10b in sertoli cells inhibit spermatogonial apoptosis by targeting KLF4. Sertoli cells deliver miR-10b to spermatogonia via exosomes. miR-10b inhibits spermatogonia apoptosis by targeting KLF4, leading to the down-regulation of the apoptosis-related gene BAX and Caspase-3.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalTableS1.xls](#)
- [SupplementalTableS2.xls](#)
- [SupplementalTableS3.xls](#)
- [SupplementalTableS4.xls](#)
- [SupplementalTableS5.xls](#)
- [Supplementalfigure1.png](#)
- [Supplementalfigure2.png](#)