Homo Sapiens (Human) microRNA 187 Expression Is Dysregulated in Testis of Non-obstructive Azoospermic Men

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Abstract

Introduction: MiRNA expression alterations are closely related to growth, proliferation, and cell development. In addition, miR-187 has been found to regulate cellular proliferation. Further, spermatogenesis is a highly complicated process which is regulated through several genes targeted by miRNAs. However, no study has completely addressed the etiology of spermatogenic impairments. Accordingly, the present study examined the expression pattern of Homo sapiens (human) microRNA (hsa-mir-187) in the testicular biopsies of infertile men with non-obstructive azoospermia (NOA) and Sertoli cell-only syndrome (SCOS) subtype in comparison with those with obstructive azoospermia (OA).

Methods: Quantitative reverse-transcription polymerase chain reaction analysis was performed to assess the expression level of hsa-mir-187 using the ΔΔCt method.

Results: Our data indicated that hsa-mir-187 was significantly up-regulated in the testis of the SCOS group (P<0.05) in comparison with the OA group.

Conclusion: In general, the findings suggest that the differential expression of hsa-mir-187 is linked with male infertility and shed more light on molecular defects that underlie spermatogenic impairments.

Keywords: Non-obstructive azoospermia, Obstructive azoospermia, miR-187, Sertoli cell-only syndrome, Testicular tissue

is critically involved in regulating cellular proliferation. Nevertheless, the role of miR-187 in male infertility pathogenesis has not been understood yet. Thus, a better understanding of the changes in miRNA expression in patients with impaired spermatogenesis might pave the way to propose new attractive therapeutic targets.

Therefore, the present study aimed to examine the expression pattern of hsa-miR-187-3p in the testis of OA and idiopathic NOA infertile patients. It is hoped that our study increases the understanding of the molecular role of miR-187 in impaired spermatogenesis fertility.

Materials and Methods

Participants

The testicular biopsy specimens of azoospermic individuals referring to the Royan Institute were obtained from patients with idiopathic NOA (Sertoli cell-only syndrome, SCOS, n = 10, the mean age = 34.8 ± 5.20 years) and patients with OA (n = 10, the mean age = 36.1 ± 5.64 years). Each patient underwent testicular sperm extraction with the goal of diagnostic biopsy for histological examination and/or assisted reproduction. Initially, azoospermia was diagnosed based on showing absent ejaculated spermatozoa in two semen analyzes examinations according to the 2010 World Health Organization criteria. Subsequently, SCOS was empirically defined as having no epididymal and/or testicular spermatozoa. OA was defined as a considerable number of mature spermatozoa sampled by testicular sperm extraction or motile spermatozoa aspirated from microsurgical epididymal sperm aspirations.

Informed consent was obtained from all patients who participated in this study. All included azoospermic men were diagnosed with primary infertility, and patients with recognized medical conditions for their infertility (e.g., the pathologies of the epididymis or vas deferens, undescended testes, varicocele, having a history of testicular tissue, the first-strand complementary DNA (cDNA) was synthesized based on showing absent ejaculated spermatozoa in two semen analyze examinations according to the 2010 World Health Organization criteria. Subsequently, SCOS was empirically defined as having no epididymal and/or testicular spermatozoa. OA was defined as a considerable number of mature spermatozoa sampled by testicular sperm extraction or motile spermatozoa aspirated from microsurgical epididymal sperm aspirations.

Real-Time Quantitative-RT Polymerase Chain Reaction

Real-time quantitative-RT polymerase chain reaction (qRT-PCR) was carried out with SYBR Green (TaKaRa, Japan) on a thermal Cycler StepOnePlus RT-PCR system (Applied Biosystems, Inc.). Next, the 10 µL PCR reaction mixture was prepared using the ExiLENT SYBR® Green PCR Master Mix (Exiqon, Denmark) following the manufacturer’s protocol. The quantitative PCR condition included the initial denaturation at 95°C for 10 minutes and 40 cycles consisting of a denaturation step at 95°C for 15 seconds, followed by an annealing step at 60°C for 30 seconds, and finally, an extension step at 72°C for 30 seconds. To ensure product uniformity, a melting curve was made by increasing the temperature from 65°C to 95°C at the end of every run. Then, PCR was conducted in triplicate, and all reverse transcriptase reactions (i.e., RT minus controls and non-template controls) were run in duplicate. In addition, the average cycle of threshold (Ct) values was computed for further analysis. Using the ΔΔCt method, data were analyzed and the U6 snRNA expression level was used as the endogenous reference.

RNA Extraction

The total RNA isolation was performed using the RiboEx reagent (GeneAll, Korea) according to the manufacturer’s protocol. Next, RNA was eluted in 30 µL of nuclease-free water. Finally, the quantity and quality of extracted RNA were measured by a NanoDrop™ 1000 spectrophotometer and denaturing agarose gel electrophoresis.

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Table 1. Primer Sequences for hsa-miR-187-3p and U6

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsa-miR-187-5p</td>
<td>RT</td>
<td>5'-CTGTATCCAGTCGACAGGCCTCGAGTTGCAGTCTGGGTAC-3'</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5'-AACGCGTTGTCGCTGCACTGGGATACGACCCCGGCT-3'</td>
</tr>
<tr>
<td>U6</td>
<td>RT</td>
<td>5'-CTGTATCCAGTCGACAGGCCTCGAGTTGCAGTCTGGGTAC-3'</td>
</tr>
<tr>
<td>(Gene ID: 26827)</td>
<td>F</td>
<td>5'-ATGACGCAAATTCGTGAAGC-3'</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5'-CAGTGGAGGCAGGCTGCTGGTA-3'</td>
</tr>
</tbody>
</table>

Note: RT: Reverse transcription; F: Forward; R: Reverse.
Statistical Analyses

The statistical analyses of this study were conducted using SPSS, version 18.0.1 (SPSS Inc., Chicago, IL), and the P value less than 0.05 was considered statistically significant. Finally, the independent t-test was utilized to compare the mean expression level of the candidate miRNA between NOA and OA groups.

Results

Infertile men participating in this study were classified as NOA (SCOS) and OA based on histological analysis. As shown in Figure 1, the relative expression level of testicular hsa-miR-187 significantly increased by 10.57-fold (P=0.04) in individuals with idiopathic SCOS compared with OA men.

Discussion

The current study assessed the expression level of testicular hsa-miR-187 in idiopathic NOA patients and OA infertile men by qRT-PCR. The hsa-miR-187 expression was significantly up-regulated in the testis tissue of the NOA group compared with OA men. It should be noted that miR-187 is speculated to have a role in the regulation of proliferation and apoptosis in various cells. Furthermore, it is strongly suggested that miR-187 reduces the ability of cancer cells to survive, inhibits their growth, and induces their apoptosis. One hypothesis is that miR-187 induces apoptosis by down-regulating Bcl-2. As an anti-apoptotic molecule, Bcl-2 is expressed in disparate cell types although it is not clear whether it acts as a target regulated by miR-187. In their study, He et al showed that miR-187 decreases the expression level of Bcl-2 in SiHa cells. They further found that apoptosis in SiHa cells is augmented as a result of Bcl-2 inhibition induced by miR-187 so that the upregulation of Bcl-2 deducts the pro-apoptotic effect of miR-187. To the best of our knowledge, the present study first reported that hsa-miR-187 is significantly linked to NOA. However, the underlying pathway through which miR-187 contributes to normal spermatogenesis needs further investigation. According to previous evidence, miRNAs modulate diverse cellular phenomena such as growth and proliferation. Moreover, spermatogenesis is among the most organized and complicated processes of cellular differentiation. Spermatogonial proliferation, as the first step of spermatogenesis, helps the stem cells to maintain and eventuate the cycle of the spermatogenetic leading to the formation of spermatozoa. Additionally, the differentiation of spermatogonia to spermatozoa is the second step of spermatogenesis which happens through a meiotic phase and sequent spermiogenesis. Similarly, apoptosis occurs during normal spermatogenesis in testis germ cells. In patients with different severity rates of spermatogenic disorders, apoptosis has been proved to be a frequent event in germ cells. For example, Lin et al approved that increased apoptosis increased in infertile men with maturation arrest and hypospermatogenesis. In other studies, transgenic mice overexpressing Bcl-2 or underexpressing Bax developed seminiferous tubule impairments with the cumulation of atypical premeiotic germ cells without mature spermatozoa, resulting in male infertility. Likewise, Amir et al reported that the altered expressions of pro-apoptotic Bax and voltage-dependent anion channel 1, and anti-apoptotic Bcl-2 are linked to oligozoospermia. However, the molecular mechanism underlying apoptosis in spermatogenesis has not been completely elucidated and requires further analysis.

Conclusion

In general, our findings indicated that testicular hsa-miR-187 is significantly up-regulated in NOA patients with complete spermatogenic failure. Accordingly, underlying pathways regarding the link between miR-187 and spermatogenesis need thorough evaluations.

Ethical Approval

This study was originally approved by the Ethics Committee of Tarbiat Modares University, Tehran, Iran (IR.TMU.REC.1395.382).

Conflict of Interest Disclosure

The authors declare there is no conflict of interests.

Authors’ Contribution

FP carried out the laboratory experiments and wrote the manuscript. In addition, HM supervised and designed the scientific work and then edited the manuscript. Further, MASG selected the patients and confirmed the clinical diagnosis. Eventually, ME analyzed the results. All authors
read and authorized the final manuscript.

Acknowledgments
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References