Introduction

Infertility, as a worldwide reproductive health problem, is estimated to affect nearly 15% of couples. Male factor contributes to 50% of couples' infertility. In 30%-40% of all cases, male infertility occurs in patients without prior infertility conditions and normal findings on physical examination termed “idiopathic male infertility.”

Azoospermia, which is referred to the lack of sperms in the ejaculate, comprises a remarkable portion of idiopathic male infertility cases and is caused by either primary testicular failure (non-obstructive azoospermia, NOA) or sperm blockage from entering the semen (obstructive azoospermia, OA).

Spermatogenesis as a highly organized, complex process of differentiation is precisely modulated by different post-transcriptional and translational events. MicroRNAs (18-22 nucleotides) modulate gene expression in different biological processes at the post-transcriptional level through interlocking to the 3’ untranslated region (3’UTR) of target mRNAs, which result in the inhibition of translation and/or mRNA degradation.

Previous studies obviously elucidated that testicular-expressed miRNAs are involved in the spermatogenesis regulation, and their expression pattern changes as the successive stages of spermatogenesis appear consecutively. To date, several genome-wide expression studies have focused on identifying testicular dysregulated miRNAs in infertile men. It is strongly suggested that hsa-miR-187, as a tumor suppressor,
is critically involved in regulating cellular proliferation. Nonetheless, 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 analyses 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 gonorrhea, 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.

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.

cDNA Synthesis

To assess the expression level of hsa-mir-187-3p in testicular tissue, the first-strand complementary DNA (cDNA) was synthesized from 1 μg of RNA using the stem-loop reverse transcription (RT) primer with the TaKaRa PrimeScript II 1st strand cDNA synthesis kit (Takara Bio, Ohtsu, Japan). All primers were designed based on Chen et al., and their details are presented in Table 1. Then, 10 μL reactions were incubated in an Applied Biosystems 9700 Thermocycler for 30 minutes at 16°C and 30 minutes at 42°C, followed by heat-inactivation for 5 seconds at 85°C and stored at 4°C. Eventually, the obtained cDNA was checked by a spectrophotometer to evaluate its concentration.

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.

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

| Gene                  | Primer | Sequence                                    |
|-----------------------|--------|---------------------------------------------|
| Hsa-miR-187-3p (MIMAT0000262) | RT     | 5’- GTCGTATCCAGTCAGGGTCCGAGGTATTCGCACTGGATACGACCAATATG -3’ |
| U6 (Gene ID: 26827)   | F      | 5’- AAGCGTCGTGTCTTGTGTTG -3’               |
|                       | R      | 5’- CAGTCCAGGGTCCGGAGGTA -3’              |

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 spermatogenic 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 accumulation 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, MASM selected the patients and confirmed the clinical diagnosis. Eventually, ME analyzed the results. All authors...
read and authorized the final manuscript.

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