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EC VETERINARY SCIENCE
Review Article

Non-Coding RNAs Role in Self Renewal of Mammalian Spermatogonial Stem Cells

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Received: November 21, 2019; **Published:** December 23, 2019

Abstract

Spermatogonial stem cells (SSCs) are the foundation for spermatogenesis which depends on the ability to self-renewal, thus any disorder of SSC biological function can result in infertility. SSC functions has important implications for understanding male infertility and its treatment by controlling self-renewal and differentiation. The fundamental mechanism underlying how SSCs acquire and maintain their self-renewal activity is of paramount importance. Several types of non-coding RNAs (ncRNAs), involving microRNA (miRNA), long noncoding RNA (lncRNA), piwi-interacting RNA (piRNA) and circular RNAs (circRNAs) play important roles in stem cell self-renewal by forming complicated epigenetic regulatory networks together with other epigenetic factors related to DNA methylation, histone methylation and acetylation. miRNAs, as critical regulator is involved in regulating self-renewal of many types of stem cell, miRNAs including miR-29b, miR-24, miR-29a, miR-199b, miR-199a, miR-27a, and miR-21 have been identified that are expressed in goat SSCs by comparing the miRNA expression profiles between CD49f-positive and CD49f-negative testicular cells using small RNA deep sequencing. Moreover, PLZF, an important transcription factor could up-regulate the expression level of CXCR4 to promote goat SSC proliferation by directly targeting miR-146a. Similarly, LncRNAs are important players in diverse cellular processes including proliferation, differentiation, apoptosis, senescence, stress response through complicated mechanisms involving lncRNA-mRNA, lncRNA-miRNA, lnRNA-DNA, lncRNA-protein. Also, some lncRNAs play critical regulatory roles in post-transcriptional and transcriptional regulation in self-renewal of SSCs. PIWI-interacting RNAs (piRNAs) are small non-coding RNAs primarily expressed in the germline cells. Furthermore, circRNAs play essential roles in various biological processes through modulating gene expression via multiple actions, such as in stem cell self-renewal and differentiation.

Keywords: *SSCs; Self-renewal; Non-coding RNA*

Introduction

Spermatogonial stem cells (SSCs) are adult stem cell population that maintains the ability to self-renew and differentiate into committed progenitors. SSCs appear postnatal and sustain throughout a male's lifespan and their biological activities establish a foundation for the sustainability of spermatogenesis [1]. As we know, that millions to hundreds of billions, of mature sperms can be produced daily in the testicular tube, it is essential that SSCs possess the capacity of self-renewal to produce large numbers of daughter cells that undergo differentiation and maintain the stem cell pool in testicular tubes, thus, the self-renewal of SSCs is crucial for spermatogenesis [2]. To date little is known regarding how SSCs acquire and maintain their self-renewal activity. However, much progress has been achieved in identifying the critical regulatory molecules that modulate SSCs self-renewal and these regulators mainly involve extrinsic growth factors,

intrinsic transcription factors, as well as epigenetic factors [3]. In addition, several signaling pathways have been shown to play important roles in controlling self-renewal of SSCs, including MEK/ERK, PI3K/Akt, Wnt/β-catenin JAK/STAT signaling [4]. In recent years, regulatory noncoding RNAs (ncRNAs), such as microRNA (miRNA), long noncoding RNA (lncRNA), piwi-interacting RNA (piRNA) and Circular RNAs (circRNAs), has been observed to be involved in regulating the self-renewal through forming an intricate regulatory network together with protein-coding gene in different types of stem cells, including embryonic stem cells (ESC), induced pluripotent stem cells (iPSCs), cancer stem cells (CSCs) and adult stem cells including SSCs [5]. In this review, we focused on recent discoveries of four types of ncRNA (including miRNA, lncRNA, piRNA and circRNAs) in self-renewal of mammalian SSC. The present work emphasis on molecular mechanisms by which ncRNAs perform their function in regulating SSC self-renewal and also, enable us to use these cells for applications in animal transgenesis, cloning and medicine.

Brief about SSCs self-renewal

Origin and self-renewal of SSCs

SSCs first arise between 0 and 6 days after birth in mammals, which are derived from primordial germ cells (PGCs) that a temporary cell population that is first observed as a tiny group of alkaline phosphatase-positive cells migrate to the genital ridge and participate in the formation of the embryonic gonad [6]. The PGCs proliferate in the original site and migrate to the future region of the gonad, where they along with somatic gonadal precursor cells, form the gonad. Once the PGCs are imbedded in testicular cord, they change morphologically and come to be prospermatogonia [7]. SSCs undergo self-renewal to maintain the stem cell pool on the one hand, but meanwhile they differentiate into sperm in mammalian testis, through three stages: mitosis, meiosis and spermiogenesis. In mitosis stage, spermatogonia can be divided into three types, including type A, intermediate, and type B cells, based upon their morphological characteristics, whereas only a small subpopulation of undifferentiated type A spermatogonia can be called SSCs. Recently, it is well demonstrated that some Aal spermatogonia can be considered as potential stem cells and may possess the potential for self-renewal in some abnormal situation such as that loss of the actual stem cells when injury or natural depletion during lifelong spermatogenesis [8].

Regulatory mechanisms of SSCs self-renewal: Stem cells are unique cells harboring self- renewal capacity such that they can refill the stem cell pool while incessantly producing the differentiated daughter cells that are required for tissue function [9]. SSCs as the only stem cell in the male body that can transmit genetic information, the tight regulation of its self-renewal is fundamental to the formation and maintenance of normal spermatogenesis [9]. Although exact molecular mechanisms underlying SSC self-renewal remain to be elucidated, many molecules primarily involving extrinsic growth factor, intrinsic transcription factor and epigenetic factor, have been identified as crucial regulators and shown to serve crucial functions in self-renewal of SSC. For extrinsic growth factors, glial cell line-derived neurotrophic factor (GDNF), a growth factor secreted by Sertoli cells in seminiferous tubules, was first identified as crucial self- renewal factor for SSCs through both *in vivo* and *in vitro* experiments. A recent study found the expression of the GDNF in STO feeder cells is sufficient to support SSC propagation without differentiation *in vitro* [10]. Fibroblast growth factor 2 (FGF2) is another growth factor that required for SSC self-renewal, which is also secreted from Sertoli cells. Both GDNF and FGF2 relies on MAP2K1 activation to drive SSC self-renewal via up-regulating expression of *Etv5*, *Bcl6b*, *Lhx1*, all of which are thought to promote SSC self-renewal. For intrinsic transcription factor, several transcription factors that promoting SSC self-renewal have been identified as the downstream targets of GDNF, including BCL6B, BRACHYURY, ETV5, ID4, LHX1 and POU3F1. Meanwhile, some GDNF-independent transcription factors also have been identified as key regulators of SSC self-renewal, such as FOXO1, PLZF, POU5F1, TAF4B, LIN28A and MYC, whose expression are not affected by GDNF [11].

For epigenetic factors, some key genes that modulating DNA methylation, histone methylation, has been demonstrated to be involved in regulation of SSC self-renewal. DNMT3L, a crucial cofactor for the *de novo* methylation of DNA, is required to delicately balance the proliferation and quiescence of SSC [12]. Interestingly, TET1, as an epigenetic regulator, not only modulates DNA methylation, also participates in histone modification to affect the ability of SSC self- renewal [13].

Non-coding RNA in spermatogonial stem cells self-renewal: Noncoding RNAs, as the novel epigenetic regulator, have been shown to play potential roles in self-renewal of SSC, further details related to research progresses in four types of ncRNA regulation of SSC self-renewal will be addressed below with emphasis, involving miRNA, lncRNA, piRNA and circRNA.

MicroRNA and self-renewal of SSCs: MicroRNAs (miRNAs) are short (19-25 nucleotides), noncoding RNAs that control the gene expression at the posttranscriptional level by binding to the 3′-untranslated regions (3′UTRs) or the open reading frames of target genes, leading to mRNA degradation or translation inhibition [14]. miRNAs, as critical regulator, have been shown to be involved in regulating self-renewal of many types of stem cell, including SSCs [15]. Several miRNAs including miR-29b, miR-24, miR-29a, miR-199b, miR-199a, miR-27a, and miR-21 have been identified that were higher expressed in dairy goat SSCs by comparing the miRNA expression profiles between CD49f-positive and CD49f-negative testicular cells using small RNA deep sequencing [16]. A study in dairy goat performed by Mu., *et al.* [17] indicated that PLZF, an important transcription factor could up-regulated the expression level of CXCR4 to promote dairy goat SSC proliferation by directly targeting miR-146a. Niu., *et al.* (2016) observed that miR-204 showed a lower expression level in dairy goat. $CD49f^{+}$ and $Thy\text{-}1^{+}$ SSCs and was involved in the regulation of dairy goat SSCs proliferation through directly interacting with the SIRT1, a gene can promote SSC proliferation and alter the expression of self-renewal and pluripotent related genes [18].

LncRNA and self-renewal of SSCs: In past decade, progresses in the mammalian transcriptome uncovered a novel class of transcripts, long noncoding RNAs (lncRNAs), which are pervasively transcribed in the genome. LncRNAs are arbitrarily defined as transcripts of greater than 200 nucleotides (nt) in length that lack functional open reading frames (ORF) and can be localized to both the nucleus and cytoplasm. Furthermore, lncRNAs are important players in diverse cellular processes including proliferation, differentiation, apoptosis, senescence, stress response through complicated mechanisms involving lncRNA-mRNA, lncRNA-miRNA, lnRNA-DNA, lncRNA-protein interaction [19], although *in vivo* functional characterization of lncRNAare still face many challenges, for instance, some of lncRNA mutant mice do not exhibit a severe or expected phenotype similar to that of *in vitro* experiment [20].

To identify lncRNAs with potential regulatory roles in SSCs, Li., *et al*. [15] identified 805 lncRNAs transcripts which were subjected to expression level changes in response to GDNF(an essential growth factor required for SSC self-renewal) through applying high-throughput RNA sequencing. A more recent study by Weng., *et al*. [21] found 473 of lncRNAs were specifically expressed in 60-day-old porcine testes containing only undifferentiated spermatogonia by RNA sequencing, indicating these lncRNA may play potential roles in pig SSC self-renewal.

Piwi-interacting RNAs and self-renewal of SSCs: PIWI-interacting RNAs (piRNAs) are a distinct class of small non-coding RNAs primarily expressed in the germline cells. These 21 - 31 nucleotide-long non-coding RNAs produced by a Dicer-independent mechanism are loaded into specific PIWI orthologs to form the piRNAs/PIWI complex and also need some other functional components such as Tudor family proteins to form a larger ribonucleoprotein (RNP) complexes to performs their function [22]. In mammals, piRNAs are expressed in two distinct phases during postnatal male germ cell development, respectively termed as pre-pachytene and pachytene piRNAs [23]. The phenotypes generated by loss of function mutations of the PIWI family genes mainly arise at two distinct stages of spermatogenic differentiation, during meiosis of spermatocytes it remains unknown whether the Piwi-piRNA pathways exerts its function in mammalian SSC self-renewal. Lee., *et al*. [24] demonstrated MILI, one of genes using *in vitro* gain of function cell culture model, including *Thy-1, Itga6, Pdgfrb, CD9* and *Hsp90a*.

CircRNAs and self-renewal of SSCs: Circular RNAs (circRNAs) are an emerging class of single-stranded RNA molecules with a covalently closed loop structure generated through a special type of alternative splicing termed backsplicing from all regions of the genome, deriving mostly from exons but also from antisense, intergenic, intragenic, or intronic regions. CircRNAs are highly stable, resistant to RNAse R and have longer half-lives compared with linear RNAs [25]. High-throughput sequencing analysis suggest that circRNAs are found to be widely expressed across species and exhibit tissue- and developmental-specific expression [26]. Emerging evidence indicates that circRNAs play essential roles in various biological processes and diseases through modulating gene expression via multiple actions, including spong-

ing microRNAs and proteins as well as regulating transcription and splicing [27,28]. Strikingly, some circRNAs have been reported to be involved in stem cell self-renewal and differentiation.

Conclusion

Spermatogonial stem cells (SSCs) are the foundation for spermatogenesis, which is dependent on the ability to self-renewal, thus any disorder of SSC biological function can result in infertility thus there is an urgent need to revealed more ncRNAs associated with SSC self-renewal to elucidate the molecular mechanisms underlying SSC self-renewal.

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Volume 5 Issue 1 January 2020