Research on Functions of Non-coding Small RNA in Germ Cells of Mammals

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Abstract. Non-coding small RNA (ncRNA) is a kind of short-chain RNA which has important functions in gene expression regulation, mainly including miRNA, siRNA and piRNA. They play the critical role in proliferation, differentiation and genome stability maintenance of germ cells of mammals through multiple mechanisms. miRNA participates in regulation of follicular development, steroidogenesis and sperm meiosis. siRNA affects maturity of oocytes by disturbing transposons and regulating microtubule kinetics. piRNA has unique functions in regulation of transposons silence and germ cell expressions. In this study, the biosynthesis of ncRNA in egg cells and sperm cells of mammals as well as its functional mechanism was reviewed systematically. Moreover, research progresses on ncRNA in germ cell development, gene expression regulation and disease development were summarized. A deep understanding on the role of ncRNA in reproductive system is not only conducive to disclose the molecular basis determined by reproductive cell fate, but also can provide new research directions and theoretical references to treat reproductive-related diseases like male infertility.

Keywords: mammals, non-coding small RNA, germ cells

1 Introduction

With the continuous development of genomics and molecular biology, non-coding RNA, especially small RNA, is attracting more and more research attention in the field of life science. Non-coding small RNA (ncRNA) exists extensively in mammal cells and plays important role in multiple physiological and pathological processes through the post-transcriptional control mechanism. In particular, ncRNA not only participates in the whole process of egg cell and spermatogenesis in the reproductive system, but also places irreplaceable role in maintaining genome stability and regulating cell cycles. Recently, the expression spectra and functional mechanisms of miRNA, siRNA and piRNA in ovary, testis and early embryonic development of mammals are disclosed gradually. This provides a new perspective for us to understand the molecular regulatory network of germ cells. This study systematically discusses biosynthesis pathways of miRNA, siRNA and piRNA as well as their functions in germ cells of mammals, aiming to summarize existing research results and lay a theoretical foundation for following studies.

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2 Biosynthesis and Functions of ncRNA

ncRNA is a kind of RNA molecules which play important role in gene expression regulation and its length is usually shorter than 200 nucleotides. According to different functions and biosynthesis pathways, ncRNA can be divided into several types. Common ncRNA includes microRNA (miRNA), small interfering RNA (siRNA) and PIWI-interacting RNAs (piRNA).

2.1 Production and Functions of miRNA

miRNA is a type of ncRNA which complements with target mRNA and inhibits its expression. miRNA mainly regulates gene expression, which is usually realized by degrading mRNA or inhibiting its translation. They are crucial in regulation of cell proliferation, differentiation, apoptosis and other biological processes. Biosynthesis of miRNA is divided into several steps. First, miRNA generates primary-miRNA (pri-miRNA) through transcription of RNA polymerase II, which occurs in the cell nucleus. Second, pri-miRNA is cut by Drosha enzyme and generates precursor-miRNA (pre-miRNA) with the hairpin structure. Third, pre-miRNA is transmits to cytoplasm through Exportin-5 proteins. In cytoplasm, pre-miRNA is further cut by Dicer enzyme to generate mature miRNA double-strand molecules with 21-23 nucleotides. Mature miRNA and Argonaute protein (AGO) combine to form the RNA induced silence complex (RISC) and develop the regulatory role[1,2].

2.2 Production and Functions of siRNA

Small interfering RNA (siRNA) is a type of ncRNA which facilitates mRNA degradation by completes with target mRNA completely. siRNA usually comes from exogenous double-strand RNA, such as virus RNA or double-strand RNA artificially synthesized in laboratory. The production process of siRNA is similar with that of miRNA, but the source and processing process are different. First, siRNA is usually introduced into cells through exogenous double-strand RNA. Second, double-strand RNA is cut into siRNA with 21-23 nucleotides in cytoplasm by Dicer enzyme. The produced siRNA and Argonaute protein combine to produce RNA induced silence complex (RISC). RISC recognizes mRNA which fully complements with siRNA and triggers its degradation or inhibiting its translation, thus finishing the gene silence process [3,4,20].

2.3 Production and Functions of piRNA

Piwi interaction RNA (piRNA) is another type of ncRNA which mainly acts in germ cells of mammals. Different from miRNA and siRNA, biosynthesis of piRNA doesn't rely on Dicer enzyme. piRNA forms Piwi/piRNA complex by combining with proteins of Piwi family and participates in maintaining gene silence as well as genome stability. The synthesis process of piRNA is relatively unique. First, the precursor of piRNA usually comes from the long single-strand RNA of genomes. These RNAs are usually

produced by transcription of specific loci. Biosynthesis of piRNA processes and produces mature piRNA through a unique "ping-pong" mechanism. This process involves two rounds of interactions and gradual cutting of RNA. Finally, the mature piRNA combines with Piwi proteins to form Piwi/piRNA complex to inhibit transposons and maintain genome stability in germ cells [5]. As show in figure 1.

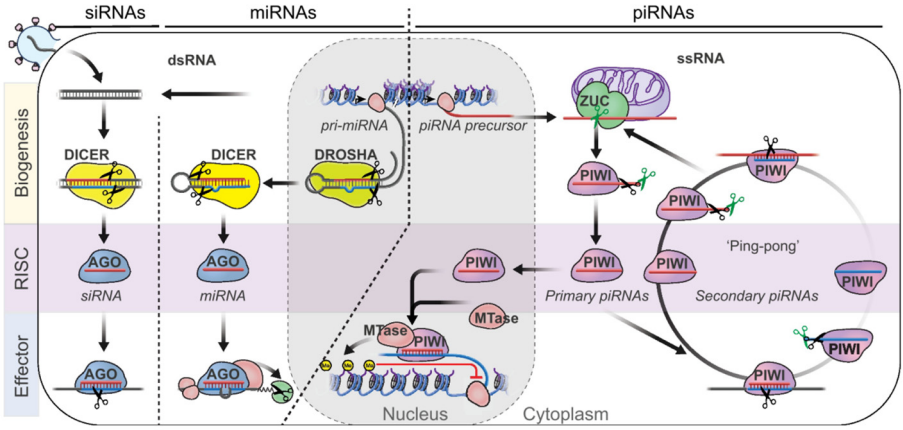

Fig. 1. Production and functions of main ncRNAs (cited from Reference [5])

3 Roles of ncRNA in Germ Cells of Mammals

3.1 Roles of miRNA in Germ Cells of Mammals

3.1.1 Roles of miRNA in Growth and Development of Egg Cells. Recent studies demonstrate that miRNA participates in follicular development, steroidogenesis, maturity of oocytes, and other processes by regulating gene expressions and signal paths. In the follicular development phase, miR-224 significantly weakens the epidermal growth factor (EGF) induced cumulocumus expansion ability through target inhibition of key gene pentraxin 3 (Ptx3) in granulosa cells (GCs), and discloses its critical role in regulation of follicular microenvironment[6]. In addition, overexpression of miR-320 in granular cells can lower synthetic efficiency of estradiol and restrict proliferation of GCs by inhibiting transcription factor E2F1 and shear factor SF-1. miR-383 further strengthens such inhibition through positive regulation of miR-320, thus influencing maturity of follicles[7]. It is important to note that miR-376a not only facilitates collection of primordial follicles to the pool in ovary of fetal rats and newborn rats by down-regulating expressions of antigen PCNA of proliferative nucleus, but also regulates the survival-apoptosis balance of egg cells. This offers a new perspective for the molecular mechanism to form primordial follicles[8]. On the signal pathway, miR-129-3p from theca cells and miR-18a-5p expressed in GCs disturb steroidogenesis and regulate expression of proteins (StAR) quickly through the target mitogen-activated protein kinase 1 (MAPK1) gene, thus regulating the activity of gonadotrophin releasing hormone (GnRH) signal pathway[9]. Meanwhile, miR-129-2-3p can accelerate follicular

degeneration by facilitating progesterone production of theca cells, and inhibiting synthesis of androgen and estradiol. miR-582-5p regulates growth and differentiation directions of follicles through the phosphatidylinositol 3-kinase - protein kinase B (PI3K-Akt) pathway[9]. Additionally, miR-34 family members (miR-34a and miR-34c) have been proved to be involved in regulation of early development of oocytes, but the specific action mechanism still needs further exploration[10].

3.1.2 Role of miRNA in Growth and Development of Germ Cells. miRNA plays an important role in regulation of meiosis, building of sperm morphology, oxidative stress response, and other procedures during spermatogenesis. According to studies, miR-34b/c and miR-449 have key regulation effects in spermatogenesis and tail formation. Gene knockout of miR-34b/c and miR-449 will lead to abnormal meiosis of spermatocytes, decreased amount of sperms and tail structural defect of mice, thus causing oligoasthenospermia or teratozoospermia[11,12]. It is important to note that the miR-34 family (miR-34a, miR-34b and miR-34c) can trigger the meiosis by activating the retinoic acid (RA) signal pathway and upregulate expression of RA synthetase. This discloses the core role of miRNA in regulation of time series of germ cell differentiation [13]. In studies on male infertility, functional diversity of miRNA is further disclosed: under oxidative stresses, the downregulated expression of miR-181b in testicular tissues will induce sperm cell apoptosis, while the abnormal high expression of miR-122a disturbs sperm chromatin remodeling process by inhibiting the level of transformational protein 2 (TNP2)[14]. Besides, exposure to microcystic toxins (MC-LR) can lead to downregulated expressions of miR-96 and thereby upregulate expressions of deleted-in azoospermia-associated protein 2 (Dazap2), thus damaging the fertilization ability of sperms[15]. The clinical studies also found that the significantly low expression of miR-15a in sperms of patients with varicocele is related with abnormal growth of oxidative stress marker HSPA1B (Heat Shock Protein 70 ku 1B), indicating that miRNA might participate in the pathogenesis of infertility by regulating the oxidative stress[16]. Moreover, the overexpression of miR-135a in spermatogonia stem cells (SSCs) of patients with cryptorchidism can destroy the self-updating ability of SSCs by inhibiting the expression of FOXO1 (forkhead box O1). This offers a new molecular target to regulate the microenvironment of reproductive stem cells[17]. These findings not only deepen the understanding on spermatogenesis regulatory network, but also lay a theoretical foundation for the diagnosis and treatment strategies of male infertility.

3.2 Role of siRNA in Germ Cells of Mammals

3.2.1 Role of siRNA in Growth and Development of Egg Cells. Endogenous siRNA plays an important role in regulating production and development of egg cells. Some studies have demonstrated that endogenous siRNA level in mice declines significantly after knockout of ribonuclease Dicer gene, resulting in the abnormal upregulation of mRNA involving in microtubule kinetics. Hence, it disturbs the correct assembly of meiotic spindle and triggers chromosome segregation barrier and meiosis retardant of oocytes[18,19]. It is important to note that deficiency of Argonaute (AGO) family

proteins (especially AGO2) may reproduce similar phenotype, which further proves the necessity of siRNA to regulate meiosis of egg cells through RNA interference (RNAi) mechanism [19]. Besides, Tam et al.[20] found that endogenous siRNA in oocytes of mice and human inhibit activity of transposons in the manner of complementary binding mode through target reverse transcription. This mechanism is crucial to maintain genome stability and normal development of oocytes. The above studies disclose that endogenous siRNA can regulate gene expressions and genome stability during maturization of egg cells.

3.2.2 Role of siRNA in Growth and Development of Sperm Cells. The role of siRNA in spermatogenesis has significant tissue specificity. Although Dicer gene mutation may cause dysfunction of testicle sertoli cells of mice and thereby lead to azoospermia and testicular degradation, mice with AGO2 gene knockout fail to show obvious testicular phenotypes, indicating the limited direct regulating effect of endogenous siRNA on spermatogenesis under physiological status. The phenotype of Dicer deficiency might be attributed to miRNA dysfunction[21]. Nevertheless, exogenous siRNA technology has become an important tool to study functions of spermatogenesis related genes for its high efficiency and specificity. For example, the self-renewal of mouse spermatogonial stem cells can be inhibited and their differentiation toward A1-A4 spermatogonial cells is inhibited through siRNA silence GfrA1 (glial cell-derived neurotrophic factor receptor A1) gene. This proves the key negative regulation effect of GfrA1 in early stage of spermatogenesis[22]. In addition, knock down of siRNA of Pard6α or Par3 gene in supporting cells will decrease the expression level of blood-testis barrier related proteins significantly, indicating that the polar protein complex based on PARD6A/PAR3 plays the core role in blood-testis barrier remodeling[22]. These studies not only disclose the functions of specific genes in spermatogenesis, but also provide strong tools to explore molecular mechanism of male infertility.

3.3 Role of piRNA in Germ Cells of Mammals

3.3.1 Role of piRNA in Growth and Development of Egg Cells. The effect of piRNA in development is not completely explicit. However, existing studies have demonstrated that piRNA may participate in formation and maturity of follicles by regulating transposons activity and gene expressions. Although no expression of MIWI proteins has been detected in adult mouse ovary, mRNA of PIWIL1 (human PIWI homologous protein) enriches significantly in primordial follicles [23]. This implies that piRNA might act in the early development stage of follicles through the post-translation regulatory mechanism. Additionally, although female mice with Miwi gene knockout are still fertile, the expression pattern of MILI (another member of mouse PIWI family) in follicle development overlap with the action time of PIWI proteins[24]. This indicates that there might be functional complementation among PIWI family members. The latest study found that piRNA in oocytes inhibits the reverse transcription activity of transposons RNA through complementary combination, thus maintaining stability of oocyte genome[25]. This mechanism is particularly important in the long-term quiescent

condition of primordial follicles. It may avoid DNA damage accumulation through silence transposons and guarantee development potentials of oocytes.

3.3.2 Role of piRNA in Growth and Development of Sperm Cells. In sperm cell development, piRNA runs through multiple key stages of spermatogenesis through dynamic regulation of transposon silence and gene expression. In male mouse testis, piRNA can be divided into three types: 1) prenatal piRNAscome from repetitive sequences. Through combination of MIWI2 and MILI, piRNA inhibits transposon activity of germ cells of embryonic phase through the "ping-pong" cycle. 2) The second type is formed by processing of mRNA 3' noncoding region. 3) The third type pachytene piRNAs come from long-chain noncoding RNA and develops explosive synthesis at about 12.5d after the birth of mice, and they take the dominant role in adult testis [25, 26]. MIWI has temporary accumulation at the 14.5~15.5 day after embryonic development and it may prevent abnormal activation of transposons during genome reprogramming of germ cells by combining prenatal piRNA silence transposons. However, the expression of MIWI2 declines quickly after birth and it cannot hardly be detected at the 4th day after birth[26]. In the postnatal phage, A-MYB transcription factor drives the synthesis of pachytene piRNAs. Pachytene piRNAs combines with MIWI proteins to form complexes. They not only silence transposons by cutting target RNAs, but also may regulate mRNA stability of spermatogenesis related genes (e.g. Tdrkh and Tdrd6) and participate in meiosis and sperm morphogenesis[25, 27]. It is important to note that functional deficiency of piRNA pathway may cause serious spermatogenesis anomaly. For example, the single site mutation in the catalytic structural domain of MILI or MIWI can cause pachytene germ cell apoptosis and male infertility, while triplet mutation of MIWI2 may cause recessive genome injury due to transposon silence functional deficiency although it cannot influence fertility significantly[26,27]. Besides, pachytene piRNA become the hub of spermatogenesis quality control due to its double functions (genome defense and regulation of gene expression). The anomaly of pachytene piRNA not only destroys meiosis homologous chromosome pairing, but also may disturb sperm acrosome formation and tail assembly, finally resulting in functional defect of sperms[25].

4 Conclusion

Researches on ncRNA in germ cells of mammals have achieved remarkable progresses. In particular, the action mechanisms of miRNA, siRNA and piRNA in follicular development, spermiogenesis and maintaining genome stability have been elaborated preliminarily. miRNA controls development processes of egg cells and sperm cells precisely through a complicated regulatory network. siRNA maintains cell homeostasis by disturbing activity of transposons and regulating expressions of meiosis related genes. piRNA becomes an important component of genome defense system through its unique "ping-pong mechanism". However, there are still many mysteries about functions of piRNA in egg cells, and the target gene regulatory networks of miRNA and siRNA are not completely analyzed. Combining with high-throughput sequencing, monoplast

technology and gene editing, future studies shall further disclose the interaction mechanism between small RNAs and their targets, explore their potential values in diagnosis and treatment of reproductive diseases, and promote deep integration between reproductive biology and translational medicine.

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