Non-coding RNAs and ovarian diseases (Review)

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Abstract. Non-coding RNAs (ncRNAs) are a diverse family of untranslated transcripts, which serve important roles in numerous biological processes. ncRNAs are emerging as major mediators of gene expression with crucial regulatory functions. Ovarian diseases have a wide variety of clinical pathological types, which have serious impacts on women's health. In this review, current studies on ncRNAs are summarized with respect to ovarian diseases. Understanding of the role of ncRNAs in ovarian diseases is currently limited; further studies on the molecular mechanisms by which abnormal expression of ncRNAs contributes to ovarian diseases will aid in the identification of ncRNAs as novel diagnostic markers and therapeutic targets for ovarian diseases.

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1. Introduction

Alterations of mRNAs have been implicated in a variety of human diseases, including cancer, cardiovascular diseases and neurodegenerative disorders, due to the changes in

protein expression levels (1-4). By contrast, ncRNAs represent a diverse family of ribonucleic acid transcripts that do not have protein-coding potential (5). The ENCODE Project established that the majority of the human transcriptomes are ncRNAs, which are involved in almost all physiological or pathological processes (6-8). With the development of genome-wide sequencing technology, more ncRNAs are being identified and characterized (9).

ncRNAs include microRNA (miRNA), small nucleolar RNA (snoRNA), small interfering RNA (siRNA), PIWI-interacting RNA (piRNA), the heterogeneous group of long non-coding RNA (lncRNA), transcribed ultraconserved region (T-UCR) and numerous other types of ncRNAs (10-13). Previously, dysfunctional miRNA-mediated regulation has been implicated in the pathogenesis of various disorders including cancer and diabetes, oncomiRs including miRNA-21 (miR-21) and miR-221/222 regulate the expression of their targets such as estrogen receptor α to modulate the progression of cancer (14-16). On the other hand, autoimmune diseases such as scleroderma have been associated with autoantibodies to protein components specific to U3 small nucleolar ribonucleoproteins (snoRNPs) (17,18); lncRNAs were closely associated with the differentiation of nerve, muscle and skin (8); and types of ncRNAs including T-UCRs, piRNAs and long intergenic noncoding RNAs (lincRNAs) were associated with human diseases. Overall, the role of ncRNAs in human diseases is gaining interest.

Ovarian diseases have a wide variety of clinical pathological types, including ovarian tumors, polycystic ovary syndrome (PCOS), premature ovarian failure (POF) and other disorders. Previous studies have demonstrated that ncRNAs were differentially expressed in ovarian diseases (19-22). For example, miR-132, miR-320, miR-520c-3p, miR-24 and miR-222 regulated estradiol concentrations, while miR-24, miR-193b and miR-483-5p regulated progesterone concentrations. In addition, miR-132 and miR-320 were expressed at significantly lower levels in the follicular fluid of patients with PCOS than in the healthy controls (20). miR-23a was highly expressed in the plasma of patients with POF and it targeted SMAD5 to regulate apoptosis in human granulosa cells (22). The present review aimed to summarize recent studies that reported the changes of several ncRNAs in ovarian diseases, which may contribute to the pathogenesis of ovarian diseases.

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2. The discovery of ncRNAs

In the early 1970s, the non-coding sequences were identified and referred as 'junk DNA' (23). Over the past decade, however, understanding of the non-coding genomes has increased (24,25). The comprehensive understanding of non-coding sequences was initiated in the 1990s, epitomized by the ENCODE consortium, which claimed complete genome sequences of numerous species (6). Subsequent to this, the ENCODE consortium identified that the majority of non-coding genomes may affect cellular and large-scale phenotypes and thus should be considered as having biochemical function. In addition, some representative ncRNAs, such as miRNAs and piRNAs, have been identified.

3. Classification of ncRNAs

ncRNAs, a diverse family of transcripts that have no potential of coding protein, include miRNA, snoRNA, siRNA, piRNA, lncRNA, T-UCR and numerous other types of ncRNAs (Table I) (10-13).

miRNAs. miRNAs, the most widely studied class of ncRNAs, act as endogenous 18-24 nucleotide long RNA molecules that recruit RNP complexes to the complementary RNAs (26). miRNAs regulate particular mRNA targets through binding to specific sequences. The outcome of miRNA binding is to inhibit target protein expression. However, the effect is not to silence mRNA expression, rather is a more nuanced effect to decrease protein levels. This can be amplified by binding multiple miRNAs to a single target, or by the targeting of multiple proteins in the same pathway (26). Numerous processes, including proliferation, differentiation, apoptosis and development, have been reported to be regulated by miRNAs (27,28).

piRNA. piRNAs are small RNA molecules 24-32 nucleotides long, which are abundant in the germline across animal species (29). piRNAs are transcribed from intergenic transcripts that are enriched in transcribed transposable elements and other repetitive elements (30-32). Previously, two piRNA-associated mechanisms have been described in *Drosophila melanogaster*: The cleavage of transposable element transcripts by PIWI proteins (33) and heterochromatin-mediated gene silencing (34).

snoRNA. snoRNAs, 60-300 base pairs (bp) long, were the first identified components of snoRNPs (35). The complexes are responsible for post-transcriptional modifications of ribosomal RNAs (rRNAs) through sequence-specific 2'-O-methylation and pseudouridylation of rRNA (35,36). snoRNAs are responsible for targeting the assembled snoRNPs to facilitate rRNA folding and stability (37).

siRNA. As 20-30 nucleotide long RNA molecules, siRNAs have emerged as critical regulators in the expression and function of eukaryotic genomes (38). Previous studies suggest widespread usage of endo-siRNAs as endogenous regulators of gene expression (39,40). siRNAs protect genome integrity in response to foreign or invasive nucleic acids including viruses, transposons, and transgenes (38). Almost all siRNAs,

whether endo-siRNAs or virus siRNAs, silence the same locus from which they are derived (38).

lncRNA. lncRNAs, a heterogeneous group of non-coding transcripts more than 200 nucleotides long, are typically transcribed and frequently spliced and polyadenylated (41,42). lncRNAs account for the majority of the ncRNAs in mammals (43). lncRNAs are predominantly localized in the nucleus and act as signals, scaffolds for protein-protein interactions, molecular decoys and guides to target elements in the genomes or transcriptomes (44). The earliest discovered lncRNAs include H19 (45) and Xist (12).

Other types of ncRNAs. Numerous other classes of ncRNA have been reported, for example endo-siRNA (22 nucleotide-long small RNAs that arise from sense-antisense RNA hybrids, pseudogene transcripts, transposable elements and mRNA exons or introns) (46), T-UCRs (DNA segments that are longer than 200 bp and are completely conserved) (47) and telomeric repeat containing RNAs (maintain the integrity of telomeric heterochromatin by regulating telomerase activity) (48). The biological functions for these ncRNAs remain poorly defined.

4. ncRNAs and ovarian development

Ovarian growth is a series of complex and coordinated processes, accompanied by significant morphological and functional changes in different follicular cells. Among the small ncRNAs associated with ovarian development, miRNAs are the most widely studied and first elucidated ncRNAs.

miRNAs and ovarian development. Dicer1 is an important RNase III enzyme that processes pre-miRNA into the shorter miRNA duplex (49). Mice with conditional knockout of Dicer exhibited increased primordial follicle pool endowment, increased degeneration of follicles and decreased ovulation rates (50). Functional studies using inhibition of miRNA biogenesis have demonstrated the occurrence of developmental arrest and female infertility in various species. Otsuka *et al* (51) reported that miRNAs were associated with the secretion of progesterone by disturbing the function of ovarian corpus. Furthermore, Hossain *et al* (52) suggested that miRNAs were involved in the regulation of steroid hormone receptors in ovarian follicle growth.

piRNAs and ovarian development. piRNAs have been described to be present in ovarian follicle cells (53-56). piRNAs may suppress transposons in the ovarian somatic cells and inhibit transposable element to transfer into the female germline in *Drosophila* (53-55).

lncRNAs and ovarian development. In multicellular organisms, lncRNAs participated in cell differentiation and individual development as promoters or inhibitors (56). In germ cell development, lncRNAs regulate the expression of specific genes and serve key roles in the complex epigenetic process.

siRNAs and ovarian development. The mouse oocyte pseudogenes siRNA system has been observed to preferentially target genes that are involved in microtubule dynamics (40).

Table I. Classification of ncRNAs.

Author, year	Name	Size	Location	Function	Illustrative example	Refs.
Mohr <i>et al</i> , 2015; Sirotkin <i>et al</i> , 2010; Jiang <i>et al</i> , 2015	miRNA	18-24 bp	Encoded at widespread locations	Targeting of mRNA and shaping the proteome of the cell	miR-15/16 miR-124a miR-34b/c miR-200	26-28
Thomson <i>et al</i> , 2009; Aravin <i>et al</i> , 2007; Aravin <i>et al</i> , 2007; Brennecke <i>et al</i> , 2007	piRNA	24-32 bp	Clusters, intragenic	Transposon repression, DNA methylation	piRNAs targeting RASGRF1 and LINE1 and IAP elements	29-32
Kiss-László <i>et al</i> , 1996; Ni <i>et al</i> , 1997; King <i>et al</i> , 2003	snoRNA	60-300 bp	Intronic	Ribosomal RNA modifications	U50, SNORD	35-37
Rinn <i>et al</i> , 2012; Flynn <i>et al</i> , 2014; Derrien <i>et al</i> , 2012	lncRNA	>200 bp	Nucleus	DNA modifications	XIST, H19	8,41,43
Carthew <i>et al</i> , 2009; Tam <i>et al</i> , 2008	siRNA	20-30 bp	Cytoplasm	mRNA silencing		38,40

ncRNAs, non-coding RNAs; miRNA, microRNA; piRNA, PIWI-interacting RNA; snoRNA, small nucleolar RNA; lncRNA, long non-coding RNA.

5. ncRNAs and ovarian tumorigenesis

miRNAs and ovarian tumorigenesis. miRNAs serve important roles in tumorigenesis by acting as oncogenes or tumor suppressor genes (57-59). The members of the let-7 family of miRNAs were observed to be lost in ovarian cancer (59). Calin *et al* (60) reported that miRNAs were frequently located in fragile regions of the chromosomes associated with the development of ovarian carcinomas.

lncRNAs and ovarian tumorigenesis. Steroid receptor RNA activator (SRA) was initially characterized as an lncRNA by Lanz *et al* in 1999 (61). SRAs were strongly upregulated in ovarian tumors (62,63), suggesting their potential role in ovary tumorigenesis. H19 acted as a type of lncRNA whose methylation imprinting was reported to be associated with the development of human benign ovarian teratomas (64). Yiya was identified as a 1.9 kb long ncRNA gene located 69 kb upstream of the transcription factor prospero-related homeobox 1 (65).

piRNAs and ovarian tumorigenesis. piRNAs have been reported to be involved in ovary tumorigenesis (21), although their specific function in tumorigenesis remains unclear. PIWI proteins have also been implicated in cancer development. For example, PIWIL2 was overexpressed in ovary tumors and inhibited apoptosis through the activation of the signal transducer and activator of transcription 3/Bcl-XL pathway (65).

snoRNAs and ovarian tumorigenesis. The potential role of snoRNAs in types of cancer, including non small cell lung cancer and breast cancer, has been reported previously (67-69), however the role of snoRNAs in the development of ovarian tumors remains unclear.

6. ncRNAs and other ovarian diseases

miRNAs and PCOS. Previous studies investigated the role of miRNAs in PCOS. Sang *et al* (20) identified seven differentially expressed miRNAs (miR-132, -320, -24, -520c-3p, -193b, -483-5p and -222) in patients with PCOS compared with healthy women. Subsequently, several groups identified differentially expressed miRNAs in rat PCOS models, including miR-9, -18b, -513-3p, -508-3p, -127-3p, -509-5p, -509-3p and -93 (70-72). The potential target genes were observed to have significantly decreased expression in the PCOS group, and included interleukin 8, synaptogamin 1 and insulin receptor substrate 2, which were associated with the PCOS phenotype (70).

lncRNAs and PCOS. Mice lacking the progesterone receptor exhibited pleiotropic reproductive abnormalities (73). In the letrozole-induced rat PCOS model, Zurvarra *et al* (74) observed increased expression levels of androgen receptor and decreased expression of the estrogen and progesterone receptors. These data indicate that steroid receptors serve important roles in the etiology of PCOS. Furthermore, several studies demonstrated that lncRNA SRA was capable of promoting the activity of these steroid receptors (75,76). Further studies are necessary to confirm the association between lncRNA SRA and PCOS.

miRNAs and POF. To understand the molecular mechanism of POF, several studies analyzed miRNAs and their target mRNAs (22,77). Profiling of differentially expressed miRNAs in POF provided novel insight into the molecular events in POF development, with the upregulation of miR-151 and miR-672 targeting expression of TNFSF10 and FNDC1, which had been demonstrated to positively regulate cell apoptosis (77).

Table II. Summary of ncRNAs involved in ovarian diseases.

Author, year	ncRNAs	Diseases	Possible role or mechanism	Illustrative example	Refs.
Yang <i>et al</i> , 2012; Kuang <i>et al</i> , 2014	miRNA	POF	Downregulation of phospholipase A ₂	miR-29a, miR-144	22,77
Sang <i>et al</i> , 2013; Roth <i>et al</i> , 2014; Liu <i>et al</i> , 2015; Lin <i>et al</i> , 2015	miRNA	PCOS	Regulation of steroidogenesis, insulin regulation, inflammation, lipid metabolism and oocyte maturation	miR-132, miR-320, miR-32, miR-34c, miR-135a, miR-9, miR-92a, miR-92b	20,70-72
Esquela-Kerschner <i>et al</i> , 2006; Hammond <i>et al</i> , 2007; Croce <i>et al</i> , 2009; Calin <i>et al</i> , 2004	miRNA	Cancer	Inhibition of the expression of cancer-associated genes, located at fragile sites involved in cancer	let-7	57-60
Zhao <i>et al</i> , 2004; Takayama <i>et al</i> , 2011	lncRNA	PCOS	Promotion of the activity of steroid receptors, enhancement of the expression of neighboring protein-coding genes	XLOC_011402, ENST00000454271	76,77
Cooper <i>et al</i> , 2009; Lanz <i>et al</i> , 2003; Miura <i>et al</i> , 1999; Yang <i>et al</i> , 2012	lncRNA	Cancer	Methylation imprinting, promotion of the cell cycle progression, modification of prospero-related homeobox 1 promoter	H19, Yiya	62-65
Yan <i>et al</i> , 2011; Lee <i>et al</i> , 2006	piRNA	Cancer	Inhibition of apoptosis	PIWII2	21,66

ncRNAs, non-coding RNAs; miRNA, microRNA; lncRNA, long non-coding RNA; piRNA, PIWI-interacting RNA.

7. Significance of ncRNAs in the diagnosis of ovarian diseases

At present, it is not possible to elucidate whether altered ncRNA expression profiles are associated with the occurrence of ovarian diseases. However, miRNAs have been isolated from the blood, saliva, urine, feces, follicular fluid and other body fluids, and secreted miRNAs remain stable in body fluids, thus may serve as biomarkers for associated diseases (78). Several serum miRNAs (miR-222, miR-29a and let-7) have been suggested to act as novel non-invasive biomarkers for ovarian diseases (59,77,79).

The technological development in the field, particularly bead-based flow cytometry, single molecule detection and massively parallel sequencing coupled with the mirage approach, may aid in the development of automated and high-speed ncRNAs profiling in the near future.

8. Conclusion and perspective

ncRNAs are highly abundant in living organisms and serve important roles in numerous biological processes. Therefore, there has been an increasing requirement to investigate the entire ncRNAomes and their biological function in further detail. In addition, aberrant ncRNA expression profiles are considered to be associated with the pathogenesis of ovarian diseases (Table II). To better understand the role of ncRNAs in ovarian diseases, future studies should focus on the molecular

Figure 1. The ncRNAs crosstalk and form an ncRNA regulatory network to modulate gene expression and function via genetic and epigenetic mechanisms. The abnormal gene expression and function then leads to a variety of ovarian diseases including ovarian cancer, PCOS, and POF. ncRNAs, non-coding RNAs; PCOS, polycystic ovary syndrome; POF, premature ovarian failure; siRNA, small interfering RNA; snoRNA, small nucleolar RNA; piRNA, PIWI-interacting RNA; lncRNA, long non-coding RNA; T-UCR, transcribed ultraconserved region.

mechanisms by which abnormal expression of ncRNAs contributes to ovarian diseases, particularly the identification of the ncRNA regulation network and the interaction between

ncRNAs and DNAs (Fig. 1). These investigations will aid in the identification of ncRNAs as novel diagnostic markers and therapeutic targets for ovarian diseases.

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