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Potential Regulatory Functions of MicroRNAs in the Ovary

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Abstract

The interactions between ovarian germ and somatic cells and expression of several intraovarian autocrine/paracrine regulators are major contributing factors in the ovary. These intraovarian mediators regulate various ovarian cellular activities including cell growth, differentiation, and apoptosis, which are critical in follicular development. Micro-RNAs (miRNAs) have emerged as key components of posttranscriptional gene expression. Recent evidence generated in mice implicates the regulatory function of miRNAs in oocyte maturation and ovarian follicular development. In the human, miRNAs may target specific gene expression in granulosa cells and participate in establishment and progression of ovarian cancer. Here, we review the currently available information on the expression and potential regulatory functions of miRNAs in the ovary under normal and pathologic conditions. Understanding the underlying mechanisms of how ovarian germ cell and somatic cell miRNAs are regulated and identifying their specific target genes and their functions may lead to the development of strategies to achieve target-specific gene regulation for the prevention and treatment of various ovarian disorders.

Keywords

MicroRNA; gene expression; folliculogenesis; ovary; polycystic ovary syndrome; ovarian cancer

The ovary maintains and nurtures the germinal cells through delicate interactions with ovarian somatic cells until oocytes mature and participate in fertilization. Detailed morphologic and biochemical information has been generated regarding various aspects of cellular communications between these cells from the neonatal period until the establishment of cyclic follicular development after puberty.^{1–5} At the molecular level, these processes are regulated by several intraovarian gene products whose precise expression is fundamental for healthy maturation of oocytes until ovulation.^{1,5–25} The establishment of primordial follicles early in the ovarian development and their subsequent development into the primary follicles has a direct consequence on the number of available oocytes throughout the female reproductive years. During this period, not only the generation and development of competent oocytes but also an optimal and timely production of sex steroid hormones is necessary for preparation of the reproductive tract tissues for fertilization, endometrial embryo implantation, and the establishment of normal pregnancy.

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NOTE ADDED IN PROOF

An article describing dysregulation of microRNAs in ovarian carcinogenesis and association with the prognosis of serous ovarian carcinoma has been published following the submission of this article.¹¹⁵

Depletion or limited pool of primordial follicles and changes in ovarian steroid production proceeds to infertility or under normal physiologic conditions of ovarian aging results in a menopausal state.^{26–28} Although controversial results from mice studies suggest the presence of a population of germ-line stem cells that may maintain follicle numbers during adult life,²⁹ in humans, it was speculated that if such germ-line stem cells exist, they could also participate in development, maintenance, and depletion of ovarian reserve. It has been proposed that if the follicles produced before birth fail to survive the process of maturity, maintenance of fertility may depend on the development of young follicles produced by germ-line stem cells, which remains to be elucidated.^{26,30}

During normal reproductive years, hundreds of resting primordial and primary follicles undergo growth initiation from which 10 to 20 antral follicles are recruited at the beginning of each normal cycle, and one is selected and proceeds to ovulation.³¹ The recruitment of growth-arrested primordial follicles into the growing follicles is complex and known to be independent of follicle-stimulating hormone (FSH) but influenced by several intraovarian factors.^{23,32–36} However, the transition from antral follicles into preovulatory follicles (Graafian follicles) is under control of coordinated actions of several hormones as well as intraovarian regulators.^{7,9–12,17,37–43} With initiation of primordial follicle growth, the oocyte begins with maternally derived gene transcription, and granulosa cells undergo rapid proliferation forming several cell layers and become the main source of sex steroid hormone production. In addition to the interaction with oocytes, granulosa cells interact with bordering theca interna, and theca externa establishes the mesenchymal-epithelial cell interactions that promote follicular communication and assist in their developmental processes. As such, the precise expression and regulation of intraovarian factors is critical to the outcome of follicular development.

The intraovarian factors act in autocrine and paracrine manners and are responsible for regulating events ranging from cell proliferation, differentiation, apoptosis, and hormone secretion. Among the key intraovarian factors, the transforming growth factor β (TGF- β) family members, bone morphogenetic protein-4 (BMP-4), BMP-8b, and BMP-2, have been identified as regulators of primordial germ cell generation.²³ In response to FSH, the granulosa cell-derived factors such as kit ligand, transforming growth factor α (TGF- α), and epidermal growth factor (EGF) activate, whereas factors such as anti-Müllerian hormone (AMH) inhibit the resting follicle growth. Oocyte-derived factors, growth differentiation factor 9 (GDF9) and BMP-15, also control follicle growth.³¹ The interaction between granulosa cells and the oocytes leading to preantral and antral follicles development involves FSH, estrogen, and androgen receptors.⁴⁴ Luteinizing hormone (LH)-stimulated theca cells produce androgens that are aromatized to estrogens by FSH-stimulated granulosa cells.⁴⁴ Estrogen production by granulosa cells is also regulated by inhibins and insulin-like growth factor 1 (IGF-1). During the later stages of follicular development, FSH-induced LH receptors on granulosa cells allow these cells to respond to both gonadotropins.⁴⁵ With the onset of the midcycle LH surge, the preovulatory follicle shifts steroidogenesis from androgen and estrogen to progesterone production during the final stages of oocyte maturation. After ovulation, granulosa and theca cells undergo luteinization forming granulosa/theca-lutein cells and a major source of progesterone and estrogen production essential for early embryonic development and embryo implantation.

In summary, it is clear that optimal expression of gonadotropins, intraovarian mediators, and their receptors that mediate their intracellular actions is necessary for normal follicular development and other ovarian functions. Alterations in the expression of these mediators also result in various ovarian dysfunctions causing infertility, polycystic ovary syndrome, and tumorigenesis. Recent evidence suggests that microRNAs (miRNAs), through transcriptional and translational regulation of their specific target genes, play a fundamental role in the outcome of all cellular and tissue activities under both normal and pathologic conditions. Only

limited information is currently available of the expression and function of miRNAs in the ovary. Here we will discuss the most recent information of the expression and possible regulatory function of miRNAs in several ovarian cellular activities, including their expression in human granulosa/cumulus cells obtained from women undergoing assisted reproduction.

miRNAs AND FOLLICULAR DEVELOPMENT

For follicular development and ovulation to proceed correctly, a coordinated and bidirectional communications mediated by a vast number of gene products is essential.^{1,7} The expression of these genes must be precise and timely. Any alteration in the expression of these genes could lead to oocyte developmental abnormalities, apoptosis, and poor cellular communication with the supporting somatic cells affecting normal follicle recruitment and development. Gene expression is a highly regulated and complex process controlled at transcriptional and translational levels. Recently, it has become evident that a group of non-protein-coding genes that transcribe a family of small RNAs also act as key regulators of gene expression stability.^{46–50} Among this family of small, non-protein-coding RNAs are miRNAs, which target gene expression stability through transcriptional repression or degradation.^{46,47,51,52}

miRNAs are ~22 to 24 nucleotides long and are transcribed from genes present in all chromosomes except Y chromosome^{47,53–55} and found in intergenic (intergenic miRNAs) and within the intron regions (intronic miRNAs) of protein-coding genes.⁵⁶ The primary transcripts, or pri-miRNAs, are several kilobases long and undergo substantial processing in the nucleus resulting in generation of a 70- to 90-nucleotide (nt) stem-loop precursor miRNA (pre-miRNA). After additional processing in the cytoplasm by Dicer, a double-stranded miRNA duplex is generated that contains 2-nt-long 3' overhangs that unwind and form a single-strand, mature miRNA.^{57–59} The mature miRNAs incorporate into the RNA-induced silencing complex (RISC) and through complementary interaction with target genes result in sequence-specific translational repression or mRNA degradation.^{53,60,61} Through this mechanism, miRNAs are considered to influence the outcome of various cellular activities under normal and disease states.

Many putative miRNAs have been identified and/or predicted in the genome of different species, including mammals. In humans, more than 580 distinct miRNA sequences have been identified, but more than 1000 have been predicted and could target the expression of protein-coding and possibly non-protein-coding genes based on the degree of their sequence homology with their target genes.^{62–64} Because the specificity of miRNAs is dictated by 6 to 7 nt that bind to the 3' untranslated region (3' UTR) of their target mRNAs, a single miRNA can potentially target hundred of genes, and a single gene could be a potential target of many different miRNAs.

To illustrate the importance of miRNAs in folliculogenesis, two recent studies uncovered an essential function for Dicer, a polymerase II enzyme responsible for miRNA processing, in regulating mouse oogenesis.^{65,66} Murchison and colleagues have reported that disruption of Dicer specifically in growing oocytes results in their inability to complete meiosis, with defects in meiotic spindle organization and chromosome congression.⁶⁵ It was also discovered that miRNAs may be involved in turnover of many maternal transcripts whose degradation may be essential for successful completion of meiotic maturation by oocytes. In wild-type mice, Dicer expression was detected mostly in fully grown germinal vesicle (GV) oocytes, a time when their development is largely driven by regulated use of maternally stored mRNAs.^{65–67} The decrease in Dicer expression, which is initiated with the onset of oocyte maturation, is common to many maternal mRNAs.^{65–67} These observations further point out the importance of Dicer in meiotic maturation of oocytes.

During the course of meiotic maturation, the oocyte is transcriptionally quiescent, which persists through fertilization and pronucleus formation until the zygotic genome becomes active.^{65–67} In addition, many miRNAs are detected in oocytes throughout the development and are considered to function as key regulators of maternally derived transcripts.^{65,66,68,69} Evidence supporting the regulatory function of miRNAs in oocytes comes from two recent studies.^{65,66} Using in vitro-matured oocytes obtained from wild-type and *Dicer*^{−/−} mice defective in miRNA processing, micro-array gene expression profiling indicated a significant difference in transcript levels between the two groups, suggesting potential consequence of loss of miRNAs' regulatory function.⁶⁵ These results also supported the findings of an earlier study that identified a set of mRNAs in the oocytes that degrade during meiotic maturation.^{70,71} Among the miRNAs identified in *Dicer*^{−/−} oocytes with potential of targeting the unregulated transcripts were mmu-miR-495, mmu-miR-126, and mmu-miR-302c*, along with six hexamers not related to any known mouse miRNAs.⁶⁵ These hexamers were seed sequences for known human miRNAs miR-570, miR-656, miR-548d, miR-496, miR-524*, and miR-576.⁶⁵

It has been further demonstrated that the maternally inherited miRNAs are functionally important for early oocyte development.⁶⁶ Using *Dicer*^{−/Flox}, *Zp3-Cre* transgenic mice with loss of most, if not all miRNAs, it was demonstrated that maternally derived *Dicer* and miRNAs in the oocyte are crucial for the earliest stages of embryonic development. The loss of *Dicer* from oocytes rendered the female mice infertile as the fertilized oocyte failed to proceed through the first cell division and blastulation.⁶⁶ These observations led to the hypothesis that in the oocyte, maternal miRNAs directly or indirectly accelerate transcript turnover during developmental processes.^{65,66} This hypothesis was further supported by demonstration of the dynamic changes in miRNA expression profiles during oogenesis using growing oocytes obtained from postnatal days 15 to 16, postnatal days 20 to 21, and of mature oocytes from adult mice.⁶⁶ The pattern of miRNA expression in these mature oocytes was essentially the same as the pattern in the zygotes, suggesting the maternal origin of miRNAs in both oocytes and the zygotes.⁶⁶ Evidence also suggests that miRNAs detected in sperm derived from testis do not participate in the transcriptional regulation of the zygote.^{68,72} Relative to metaphase II oocytes, the sperm-borne miRNA levels were low, and fertilization did not alter the oocyte miRNA profile, which should have incorporated the most abundant sperm-borne miRNAs.⁶⁸ Additionally, co-injection of oocytes with sperm heads plus anti-miRNAs to suppress miRNA function did not perturb pronuclear activation, whereas nuclear transfer by microinjection altered the miRNA profile of enucleated oocytes.⁶⁸ The authors suggested that sperm-borne miRNAs play a limited role, if any, in mammalian fertilization or in early preimplantation development.^{68,72}

The miRNAs detected in mature mouse oocytes include miR-30, miR-16, let-7, and miR-17–92 families.⁶⁶ The miR-17–92 cluster and let-7 family are the most abundant maternal miRNAs detected in the zygote, and their profile displayed a dynamic regulation during oogenesis and early embryo development.⁶⁶ The miR-17–92 cluster has been shown to function in several cellular activities including cell-cycle regulation.^{51,52} The abundance of the miR-17–92 cluster, which is inherited by the zygote, increases during oogenesis and increases again after the two-cell embryo stage.⁶⁶ The expression patterns of some of the key genes identified during oogenesis,⁷³ including *Oct4*, *Fragilis*, *Stella*, *C-mos*, *Bnc1*, *H2AX*, *H1foo*, *SCP3*, *Nobox*, *Gata4*, and *RFPL4*, were unaffected in growing oocytes at postnatal days 15 to 16.⁶⁶ However, mature ovulated oocytes obtained from *Dicer*^{−/Flox}, *Zp3-Cre* females that were mated with vasectomized males displayed a higher level of *C-mos* and *H2AX* expression.⁶⁶ These results provided direct evidence for the role of *Dicer* and requirement for miRNA processing in mature oocytes and the zygotes.^{65,66}

The biological implication of these miRNAs in human oogenesis is speculative, nevertheless nearly all the miRNAs identified are conserved in closely related species, or many have homologs in distant species, implying that their functions are conserved throughout the evolution.^{47,74} As such, it is possible that identical or similar maternally derived miRNAs also participate in regulation of oocyte and maternal-to-zygotic transcripts in humans. Whether alteration in maternally derived miRNA results in oocyte developmental failure in humans as seen in conditionally altered Dicer gene in mice is unknown. However, transcriptional profiling of oocytes from polycystic ovary syndrome and from women with normal ovarian function^{75–79} implies a potential association between the level of expression of these transcripts and regulatory function of miRNAs. Currently, the regulatory function of miRNAs in gene expression stability of granulosa and theca cells under physiologic and disease states remains to be elucidated.

miRNAs AND REGULATION OF SOMATIC CELL-CYCLE PROGRESSION AND APOPTOSIS

Based on current information in the database, many putative miRNAs have been identified and/or predicted in the genome of different species.^{56,63} These miRNAs have been predicted to regulate up to a third of protein-coding and possibly non-protein-coding genes.^{62–64} Despite the rapid progress in miRNA identification, uncovering their target genes has been challenging. To date, 70 to 100 mammalian mRNAs have been demonstrated to be direct targets of an individual miRNA.⁸⁰ The existing challenge in target identification, at least in part, is due to the binding of miRNAs to their target genes that might not result in alteration of the level of target gene expression.^{81,82} Rather, the interaction may induce translational regression that appears to be cap and polyA-tail dependent during initiation.^{83,84} It is also unclear whether altered or aberrant expression of miRNAs is the cause or the consequence of changes in cellular activities that leads to various pathologic disorders.⁴⁶ As such, validating each gene(s) targeted by these miRNAs and elucidating the underlying mechanism of how and why their expressions and functions are dysregulated in disease versus normal state may have potential implications in understanding the pathogenesis of several ovarian disorders.

Accumulated functional analysis has focused on regulatory actions of many miRNAs on gene expression involved with tumorigenesis and cancer progression.^{55,59,60,85,86} Recent reports also illustrate the influence of miRNAs on regulation of genes involved in cell growth, differentiation, apoptosis, and inflammatory and immune response under normal physiologic conditions.^{85,87–91} These cellular processes are critical to folliculogenesis and transformation of granulosa/theca cells into luteinized cells followed by their demise, which are regulated by hormones as well as a vast number of intraovarian regulators whose expression may be the target of miRNA regulatory function.

The potential regulatory function of miRNAs in ovarian gene expression has been illustrated in a recent study in mice.⁹² Sequencing of ~400 small RNA complementary DNA (srcDNA) clones from the ovarian libraries of 2-week-old and adult animals identified 122 miRNAs, of which 15 miRNAs were unique.⁹² These novel ovarian miRNAs (miR-ov) were abundantly expressed in the ovary with miR-ov1, -ov5, -ov7, -ov10, -ov12, and -ov14 and were ubiquitously expressed in multiple tissues, with miR-ov13 exclusively expressed in the ovary, testis, and uterus.⁹² Although 11 of these 15 unique miRNAs were detected in the ovary and oocytes, miR-ov4, -ov7, -ov9, and -ov15 were not detectable in the oocytes.⁹² The biological significance of lack of expression of these miRNAs in the oocytes is not known but could be related to the fact that oocyte miRNAs are maternally derived and protected from further regulatory function.

Using ovarian granulosa/cumulus cells obtained from women undergoing oocyte retrieval for assisted reproduction, we studied the expression of several miRNAs in these cells.^{93,94} These miRNAs included miR-23a, miR-23b, miR-542-3p, miR-211, and miR-17-5p, which are predicted to target a large number of genes, including cyclooxygenase-2 (COX-2), IL-1 β , steroidogenic acute regulatory protein (StAR), CYP-19A1 (aromatase), and estrogen receptor (ER) ER β .^{93,94} COX-2, IL-1 β , StAR, aromatase, and ER are among the key regulators of ovarian sex steroid biosynthesis and other ovarian physiologic functions. The biological relevance of these observations to normal granulosa cells during growth and differentiation is unclear because the granulosa cells used in our study were derived from women undergoing assisted reproduction. Although these results provide evidence for the expression of miRNAs in specific ovarian cells, their direct participation in regulating the expression of specific genes involved in folliculogenesis as well as granulosa/theca cells proliferation, differentiation, and their influence in steroid biosynthesis awaits further investigation.

miRNAs AND OVARIAN PATHOPHYSIOLOGY

Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility and affects 7 to 8% of reproductive-age women.⁹⁵ A hallmark of PCOS is excessive theca cell androgen secretion, which is linked to the symptoms of PCOS. It is often characterized by arrested follicle development at antral stage and is frequently associated with insulin resistance.^{31,79,96} It has also become evident that altered expression or inappropriate signaling mechanisms of various intraovarian factors accounts for pathogenesis of PCOS.^{31,79,96} The link between the endocrine and intraovarian regulators has been further illustrated in several gene expression profiling studies in oocytes and ovarian tissues obtained from patients with PCOS. When assisted reproductive technologies are used in PCOS patients,^{31,79,96} there is increased rate of obtaining immature oocytes along with high rates of fertilization failure.^{92,97} Thus, to optimize developmental competence of oocytes obtained from these patients, the molecular environment determining follicle growth and oocyte development must be defined to allow new therapeutic strategies.

Aberrant expression of miRNAs has been associated with several disorders, including oocyte developmental abnormalities and fertilization failure.^{65,66} Gross analysis of the transcripts profiled in oocytes of PCOS patients compared with those of women with normal fertility suggests that a large number of genes could be potential targets of miRNA regulatory function. Annotation of the data demonstrated that a subset of these genes was associated with chromosome alignment and segregation during mitosis and/or meiosis.^{96,98,99} In ovarian granulosa/cumulus cells obtained from women undergoing oocyte retrieval for assisted reproduction, we found distinct difference in relative expression of miR-23a, miR-23b, miR-542-3p, miR-211, and miR-17-5p, and few of their predicted target genes, COX-2, IL-1 β , StAR, CYP-19A1, and ER β in the PCOS group compared with male factor group or egg donors.^{93,94} Although detailed studies are needed to establish the biological relevance of this observation, it appears that the expression of miRNAs in the ovarian supporting cells may have a direct regulatory effect on the expression of specific genes involved in folliculogenesis as well as granulosa/theca cells functions associated with PCOS and possibly other ovarian disorders.

Recent evidence indicates that several miRNAs may influence the expression of genes involved in metabolic disorders such as type 2 diabetes mellitus and insulin resistance.^{100,101} For instance, miR-124a and miR-96 have been identified as novel regulators of the expression of proteins critical in insulin exocytosis and in the release of other hormones and neurotransmitters.¹⁰² Expression profiling of miRNAs in normal and Goto-Kakizaki rats with symptoms of type 2 diabetes mellitus identified several unregulated miRNAs relative to

normal, including miR-29, miR-29a, miR-29b, and miR-29c.¹⁰⁰ The results suggested that overexpression of miR-29 family by targeting specific genes such as insulin-induced gene 1 (INSIG1) and caveolin 2 (CAV2) could lead to insulin resistance.¹⁰⁰ As such, altered expression of miR-29 family and its specific target genes involved in folliculogenesis in PCOS patients could result in ovarian dysfunction associated with this patient population.

miRNAs and Ovarian Cancer

Ovarian cancer is the fifth leading cause of cancer death in women in the United States.¹⁰³ In recent years, miRNA expression profiles have been used to differentiate the molecular characteristics of various tumors from their corresponding normal cells and tissues. A considerable number of miRNAs were identified and/or predicted to display aberrant expression patterns in malignant tumors of various systems, such as several types of lymphomas and gastric, lung, breast, pancreatic, pituitary, cervical, and ovarian cancers, compared with their corresponding normal cells and tissues.^{104–106} Analyzing the pattern of miRNAs expression in multiple tumors has indicated their developmental lineage, differentiation states, and has been found helpful in assessing whether the tumor is poorly differentiated or not.¹⁰⁷ Although direct functional relevance of the changes in expression of miRNAs and various aspects of cancer biology remains to be elucidated, the current evidence suggests that miRNAs through regulation of oncogenes and tumor suppressor genes may influence the outcome of malignant transformation. Moreover, 50% of miRNA genes are frequently located at fragile sites or common breakpoint regions.^{104,108,109} Given the potential function of miRNAs to regulate the expression of multiple genes, alteration of the expression of a single miRNA, or a miRNA cluster, as a result of chromosomal rearrangement may have significant consequences leading to neoplastic transformation.

A recent study comparing the expression of miRNAs in ovarian cancer tissues and normal ovary has identified an altered expression of a significant number of miRNAs in ovarian tumors. Among the most significantly overexpressed miRNAs in ovarian cancer included miR-200a, miR-141, miR-200c, and miR-200b. However, miR-199a, miR-140, miR-145, and miR-125b1 were among the most down-modulated miRNAs. Notably, altered expression of miR-125b has also been reported in breast cancer.¹¹⁰ Furthermore, miR-145 and miR-199a have recently been shown to be down-modulated in other tumors, and miR-140 has been reported to be deleted in ovarian carcinoma. Interestingly, miR-140 gene is located at chromosome 6q22, a fragile region often deleted in ovarian tumors, and it is predicted to target important genes cytoplasmic Src-related tyrosine kinase (c-SRK), matrix metalloproteinase 13 (MMP13), and fibroblast growth factor 2 (FGF2).¹¹⁰ The products of these genes are known to regulate several cellular activities including tumor invasion and angiogenesis.

MicroRNA signature of ovarian tumors may also distinguish these tumors based on their histologic subtypes such as serous, endometrioid, mucinous, and low- and high-grade malignancies, all showing variable clinical manifestations and underlying molecular signatures.¹¹⁰ For instance, the endometrioid tumors displayed four most significantly up-modulated miRNAs (miR-200a, miR-200b, miR-200c, and miR-141), with miR-222 identified among downregulated miRNAs. Notably, miR-212, which is downregulated in serous cystadenocarcinoma, has as putative target Wilms tumor 1 (WT1), which is overexpressed in this subgroup of ovarian cancers. miR-212 also targets the expression of breast cancer 1 (BRCA-1), which is mutated in hereditary ovarian cancer.^{110–112} Interestingly, poorly differentiated carcinomas displayed a different pattern of miRNA expression compared with that of normal ovary. Among these miRNAs, miR-373 has been described to function as an oncogene in testicular germ cell tumors.¹¹⁰ Other studies assessing the expression of miRNAs in ovarian tumors identified several miRNAs with altered expression of miR-214, miR-199a*, miR-200a, miR-100, miR-125b, and let-7 cluster compared with that of controls.¹¹³ Among

these miRNAs examined, miR-214 induced cell survival and cisplatin resistance by targeting the expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and activation of protein kinase B (Akt/PKB) pathway.¹¹³ PTEN is a tumor suppressor gene frequently inactivated in several cancers, and its altered expression and activity have been associated with other disorders such as inflammation and fibrosis.¹¹⁴ In human ovaries, the expression of PTEN and the phosphoinositide-3 kinase (PI3K/Akt pathway have been demonstrated and appear to be involved in the regulation of proliferation and differentiation of granulosa cells. In summary, it appears that a selective number of miRNAs expressed in a tissue-specific manner play key roles in both normal and pathologic ovarian activities by targeting the expression of specific genes.

CONCLUSIONS

Since the discovery of miRNAs a few years ago, the field of miRNA research has evolved rapidly. Various studies, more specifically during the past 3 years, have provided strong evidence for widespread expression and regulatory functions of miRNAs in gene expression under normal physiologic and disease states. Through their regulatory function on gene expression, miRNAs have now been recognized as key regulators of various cellular activities, including cell proliferation, differentiation, and cell death. More specifically, changes in the expression of several miRNAs have been correlated not only with normal developmental processes but also cellular transformation and tumorigenesis in all types of cancers.

As illustrated here, the available data with respect to the expression and potential regulatory function of miRNAs on their target genes in reproductive tract tissues including the ovary is limited to a few studies. As such, our discussion of the existing data on the expression and potential role of miRNAs in the ovarian cellular activities, specifically in the human, was speculative in nature. Undoubtedly, growing evidence using both in vivo and in vitro systems will lead to the identification of a specific group of miRNAs that may act as key regulators of genes implicated in various aspects of ovarian physiology. Indeed, a single miRNA can potentially target hundreds of genes, or a single gene could be a potential target of many different miRNAs. Although experimental validation of these predicted target genes is a rather complex task, this information is necessary for understanding the biological actions and functional mechanisms of specific miRNAs relevant to the ovary. Use of animal models would allow us to elucidate the specific ovarian expression and function of miRNAs under normal conditions. Because of conservative nature of miRNAs among various species, data generated from animal models could reflect the nature of their expression, regulation, and functions in human ovarian cellular activities. Such understanding is critical to identify the important target genes whose products contribute to the underlying mechanism of ovarian activities that result in infertility or subfertility, as well as ovarian cancer. Some of the immediate areas for investigation include elucidating the developmental expression and regulation of miRNAs in theca and granulosa cells and differentiation into luteal cells; whether miRNAs are differentially regulated by gonadotropins and intraovarian mediators; and how and by what mechanisms miRNAs influence the translational stability of target gene expression? Continued efforts to elucidate the function of miRNAs, more specifically those related to ovarian cellular activities, should reveal novel insights in how their target genes are regulated under normal physiologic conditions, and the results could have prognostic or therapeutic implications for the management of patients with various ovarian dysfunctions including PCOS and cancer.

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ABBREVIATIONS

3' UTR	3' untranslated region
Akt/PKB	activation of protein kinase B
AMH	anti-Müllerian hormone
BMP	bone morphogenetic protein
BRCA1	breast cancer 1
CAV2	caveolin 2
COX-2	cyclooxygenase 2
c-SRK	cytoplasmic Sky-related tyrosine kinase
EGF	epidermal growth factor
ER	estrogen receptor
FGF2	fibroblast growth factor 2
FSH	follicle-stimulating hormone
GDF	growth differentiation factor
GV	germinal vesicle
IGF	insulin-like growth factor
IL-1β	interleukin-1 β
INSIG1	insulin-induced gene 1
LH	luteinizing hormone
MMP13	matrix metalloproteinase 13

nt	nucleotide
PCOS	polycystic ovary syndrome
PI3K	phosphoinositide-3 kinase
PTEN	phosphatase and tensin homolog deleted on chromosome 10
RISC	RNA-induced silencing complex
srcDNA	small RNA complementary DNA
StAR	steroidogenic acute regulatory protein
TGF-α	transforming growth factor α
TGF-β	transforming growth factor β
WT1	Wilms tumor 1

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