MicroRNAs and Their Role in Gynecological Tumors

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Abstract: There have been only few events in the history of molecular biology that could be compared to the discovery of microRNAs and their role in cell physiology and pathology. MicroRNAs are small, single-stranded, noncoding RNAs composed of 19–25 nucleotides (~22 nt), which have been proven to regulate gene expression at the posttranscriptional level. The regulatory function of microRNAs was demonstrated in normal and diseased conditions. In particular, it has been linked to cell cycle regulation, cell proliferation and differentiation, inflammatory response, and apoptosis. Altered expression profiles of microRNA have been observed in many pathologies, including diabetes, rheumatoid arthritis, and several cancers. To date, more than 700 human microRNAs have been identified and in silico-based analyses estimate at least 500 more to be identified. The purpose of this review is to present the current perspective on microRNAs structure and biogenesis as well as their contribution to the etiopathogenesis of gynecological tumors. We discuss results of the recent publications that indicate possibilities of microRNAs use as novel markers for tumors screening, early diagnosis, and treatment monitoring. The possible utilization of microRNAs as prognostic factors and specific therapy targets is also reviewed. Med Res Rev, 31, No. 6, 895–923, 2011

Key words: microRNA; ovarian cancer; endometrial cancer; cervical cancer; leiomyoma

1. INTRODUCTION

Increasing incidence and mortality of cancer worldwide has caused the search for novel diagnostic and therapeutic agents to become a top priority of modern science. Despite enormous efforts that have been made in the field of cancer research, the knowledge concerning etiology and pathogenesis of multiple malignancies, including gynecological neoplasms, is

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still incomplete. A new insight to cancer etiopathogenesis occurred after the discovery of microRNAs, which have been linked to the regulation of gene expression at the post-transcriptional level.

MicroRNAs are small, single-stranded, noncoding RNAs composed of 19 to 25 nucleotides (~22 nt), which have been independently discovered in the nematode Caenorhabditis elegans by Lee et al. and Wightman et al. in 1993.[1,2] But it was not until the beginning of 21st century that numerous studies confirmed the presence of multiple microRNAs in different organisms and proved their essential role in cells' metabolism. The regulatory function of microRNAs has been linked to cell proliferation, cell cycle regulation, differentiation, inflammatory response, and apoptosis.[3–9] Altered expression profiles of microRNAs have been observed in many pathologies, including diabetes, rheumathoid arthritis, cardiovascular diseases, and several cancers.[10–15] Multiple studies performed over the last few years led to a huge increase in the data describing microRNAs biology. To date, 721 human microRNAs have been sequenced and are detailed in the Sanger database (as of November 28, 2009: http://microrna.sanger.ac.uk/sequences/).[16] It has been estimated that at least 500 more are to be identified.

This review presents the current knowledge regarding the microRNAs' contribution to etiopathogenesis of gynecological tumors.

2. MICRORNAS STRUCTURE AND PROCESSING

MicroRNAs encoding genes comprise approximately 1–2% of the human genes.[17] Mature microRNAs derive from precursors called pri-microRNAs composed of hundreds or thousands of nucleotides. Sequences encoding microRNAs precursors are located in different parts of nuclear DNA and may constitute mono- or policistrone transcriptional units, e.g. pri-miR-21, pri-miR-17-92-1, or can be located within introns; pri-miR-10b or exons; or pri-miR-198 of protein encoding genes.[18–21]

Pri-microRNAs are transcribed mainly by polymerase RNA II; however, polymerase III has also been documented to transcribe a number of microRNA genes.[22,23] Afterwards, they are cleaved by endonuclase Drosha and cofactor DGCR8. The newly created pre-microRNAs, a ~60 nucleotides stem-loop molecules, are transported from the nucleus to the cytoplasm and further processed by the Dicer enzyme.[24] MicroRNA biogenesis pathway is schematically presented and described in Figure 1.

It has been proven that microRNAs alter cell metabolism through gene expression regulation which takes place by means of two mechanisms. Mechanism that occurs mainly in animals consists in binding the microRNA–RISC complex to 3′ untranslated region of mRNA, does not require perfect complementarity, and induces inhibition of translation at the initiation or elongation phase.[25] It has also shown that animal microRNAs have ability to cleavage mRNA by a direct mechanism dependent on their interaction with 3′ untranslated regions.[26]

Different mechanism of microRNA action has been observed in plants, in which mRNA degradation by Ago2 protein depends on binding of microRNA–RISC complex to the open reading frame with perfect or almost perfect complementarity.[27]

Mechanisms of microRNA-dependent gene expression regulation are schematically presented in Figure 2.[25–33]

Until recently, inhibition of posttranscriptional mRNA processing was assumed to be the only possible way of microRNA-dependent gene expression regulation. Unexpectedly, recent studies revealed that microRNA could activate translation during cell cycle arrest.[4] Though such a mechanism has only been shown to be operative for a limited number of

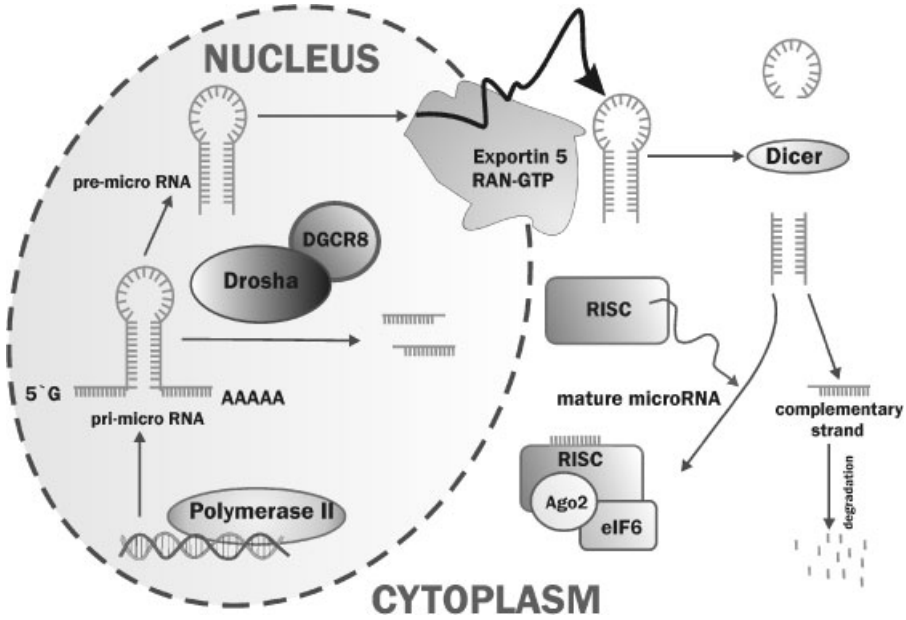

Figure 1. Biogenesis of microRNA. The process starts with pri-microRNA synthesis by polymerase II or polymerase III. Pri-microRNA undergoes cleavage by a complex Drosha–DGCR8 complex into ~60 nucleotides pre-microRNA stem-loop molecule and single-stranded RNA fragments. Pre-microRNA is then transported to the cytoplasm by exportin 5 and protein Ran-GTP where it is further modified by RNAs III enzyme called Dicer into double-stranded molecules composed of ~22 nucleotides. Subsequent junction with RNA-induced silencing complexes (RISC) induces unwinding of the double-stranded molecule into single-stranded microRNA, at the same time the complementary strand is degraded. eIF6 and proteins belonging to argonaute family, Ago1, Ago2, Ago3, and Ago4, constitute important components of such newly created complex.

microRNAs and in limited context, it changes our understanding of the microRNAs' regulatory potential. The complexity of microRNAs' actions is further enhanced by the fact that each of those small molecules can affect expression of more than 200 genes and, on the other hand, particular mRNAs may be regulated by several different microRNAs.[34]

3. METHODS OF MICRORNAS DETECTING AND PROFILING

Since microRNAs discovery, various methods of their detection and profiling have been developed. MicroRNAs identification in tissues can be performed using high-throughput methods, such as oligonucleotide microarray, liquid bead-based array, and real-time PCR-based array. Results acquired using those techniques need to be, however, further validated by more sensitive and specific methods, including Northern blot, real-time PCR assays, and in situ hybridization.[35–40] The first real-time PCR assays for microRNA detection were designed in 2004 and 2005 by Schmittgen et al. and Chen et al., and provided tools for profiling both precursor and mature microRNAs.[39] A new approach to microRNA expression has been recently presented by Wyman et al., who employed massively parallel pyrosequencing, in order to overcome limitations attributed to microarray studies, which include incomplete coverage of known microRNAs, inability to discover and profile novel microRNAs, and difficulties in distinguishing between molecules differing by only one or two nucleotides. This approach enabled discovery of 6 novel and 39 candidate microRNAs in the ovarian cancer study performed by the aforementioned research group.[41] Mirage, a technique which combines hybridization and polymerizations steps, constitutes another method that

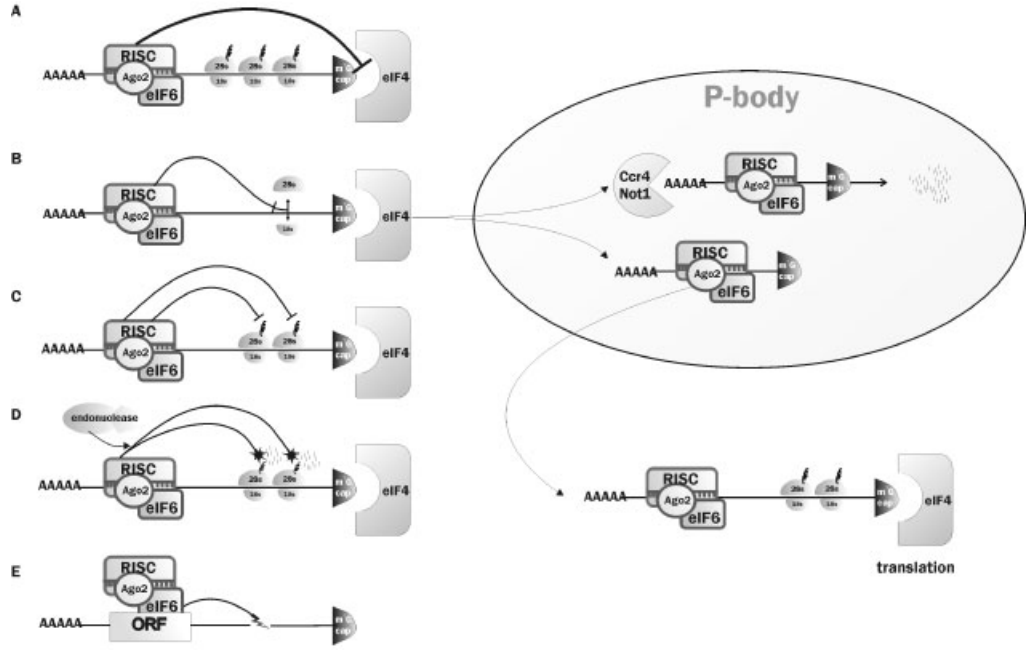

Figure 2. Mechanisms of gene expression regulation by microRNA. Inhibition of translation: (**A**) at the initiation phase by competition between RISC and the translation initiation complex protein eIF4 for 5′-terminal m^7G cap structure binding; (**B**) at the initiation phase by eIF6 protein-dependent blockage of ribosomal subunits bonding on mRNA; a ribosome-free mirRNA: mRNA complex enters a P-body were Ccr4/Not1 deadenylase shortens poly (A) tail which results in mRNA degradation; (**C**) at the elongation phase through a decrease in elongation rate; (**D**) at the elongation phase through a decay of the nascent protein. (**E**) Mechanism resulting in degradation of mRNA induced by Ago2 protein endonuclease activity that occurs mainly in plants and involves combining the microRNA–RISC complex to open reading frame (ORF) with perfect or almost perfect complementarity.

not only profiles known microRNAs but also has the capability to identify new ones as well.[42] Finally, nanotechnology methods has lately been utilized allowing detection and amplification of microRNAs in femtomolar range.[43,44]

Profiling of mature microRNAs is generally preferred in most study designs; however, if identification of mature microRNAs is impossible, methods of pre-microRNAs detection may reveal a distortion of their biogenesis due to improper processing by Dicer or Drosha. Thus, experiments designed to profile both pre- and mature microRNAs are capable of unraveling possible causes of microRNA expression alterations.[39]

Until recently, microRNA profiling studies were conducted using RNA samples derived exclusively from tissues. Recently, an interesting study by Chen et al. was published proving the presence and possibility of microRNA detection in serum and other body fluids, including urine, tears, ascetic and amniotic fluid both in normal and diseased conditions.[10] The study also revealed that microRNAs comprised the largest fraction of small RNAs in human serum, were stable and resistant to harsh condition, and that their level was reproducible and consistent among healthy individuals. Additionally, the authors found that microRNAs, isolated from the serum of cancer patients, differed compared to healthy objects and were not derived from circulating blood cells.[10] Similar results were obtained by Lodes et al., who determined that an amount of microRNA derived from 1 ml of human serum was sufficient to obtain reliable results, using a fluorescence or electrochemical detection-based microarray.[45] These observations combined with the results of the studies performed among patients with diffuse large B-cell lymphoma, prostate cancer, and ovarian cancer, in which serum microRNA profiles were capable of distinguishing between cancer and healthy

individuals, implicate microRNAs as potential serum-based biomarkers.[46–48] Stability of microRNAs extracted from formalin fixated tissues was also proven by several studies. Xi et al. indicated stable expression level of microRNAs derived from formalin fixed samples over a 10 year period.[49] Moreover, distinct microRNA profiles expressed in fresh frozen and formalin fixed paraffin-embedded samples have been proven to distinguish between normal and diseased tissues in several cancers.[40] The possibility of microRNA detection in archival material offers a unique opportunity to correlate patients' background and follow-up data with microRNAs expression profiles.

Methods that allow detection and profiling of microRNAs need to be accompanied by studies that can provide information on the biological effects of particular microRNAs. Identification of microRNA targets is the first and indispensable step in the process of unraveling their regulatory function in normal and diseased conditions. As experimental methods are strenuous and time consuming, bioinformatic approaches to unravel potential microRNA targets have been developed. Several databases that allow prediction of possible microRNA targets are currently available. Algorithms applied by such databases are based on the complementarity between microRNA and its target sequence, thermodynamics of the microRNA–mRNA duplex, and evolutionary conservation of the microRNA- and mRNA-binding sites. Discovering, identification, and sequencing novel microRNAs that were initially performed experimentally have led to the conclusion that the number of possible microRNA sequences was limited. Such proposal was, however, verified with the recent application of methods based on deep sequencing of small RNA libraries.[17,50,51]

4. MICRORNAS EXPRESSION PROFILING STUDIES IN GYNECOLOGICAL NEOPLASMS

The potential role of microRNAs in cancer has been implied by several studies. A significant number of microRNAs has been shown to be downregulated in various malignancies, suggesting their possible role as suppressor of carcinogenesis.[15,52–54] On the other hand, expression of some microRNAs was found to be increased in cancer indicating that they may act as oncogenes. Examples of such microRNAs include the miR-17-92 cluster overexpressed in B-cell lymphoma and lung cancer and miR-21 upregulated in colorectal cancer.[55,56] Additionally, functional studies have proven that targets of several microRNAs include well known tumor suppressors and oncogenes.[30,57] For example, let-7, which is downregulated in several cancers, targets well documented oncogene RAS, and miR-15 and miR16 both decreased in chronic lymphocytic leukemia's target, antiapoptotic factor BCL2.[58,59]

Given that microRNAs act mainly through inhibition of translation, changes in microRNAs levels may potentially alter expression of their target oncogenes and tumor suppressor genes, thus influencing the process of carcinogenesis.

The potential of microRNA utilization in clinical practice includes screening and early diagnosis, possibility of classifying tumors, tracing the origin of metastases, and poorly differentiated malignancies, as well as prediction of survival and response to treatment.[60,61]

Various and distinct microRNA expression profiles have been identified in tissue samples of several cancers, including gynecological malignancies. Tables I and II present a summary of microRNAs profiling studies in four common gynecological tumors.

A. MicroRNA Studies in Ovarian Cancer

Ovarian cancer is the second most common malignancy of the female reproductive tract and the fifth leading cause of cancer mortality, responsible for more than 140,000 deaths each

Table I. Summary of the MicroRNAs Expression Profiling Studies in Ovarian Cancer

Authors	Material and methods	*n*	MicroRNAs altered in the study The most significant expression alteration
Iorio et al.[63]	Sixty-nine cancer tissue samples and 5 cell lines vs. 15 normal ovarian tissue sections; microarray platform, quantitive RT-PCR, Northern blot	29	Upregulated: miR-200a, miR-200b, miR-200c, miR-141. Downregulated: miR-199a, miR-140, miR-145, miR-125b1
Yang et al.[74]	Ten serous ovarian cancer tissues vs. 10 HOSE cell lines; microarray platform, Northern blot	36	Upregulated: miR-214, miR-199a, miR-200a, miR-424, miR-302d, miR-320, miR-200a, miR-29a. Downregulated: miR-493-5p, miR-494, miR-125b, let-7a,b,c, miR-100
Dahiya et al.[76]	Thirty-four ovarian cancer tissues vs. HOSE-B cell line; microarray platform	56	Upregulated: miR-221, miR-146b, miR-663, miR-29c, miR-29a, miR-100, miR-199a, miR-99a, miR-508, miR-296, miR-494
	Ten ovarian cancer cell lines vs. HOSE-B cell line; microarray platform	28	Downregulated: miR-21, let-7e,f, miR-106b, miR-122, miR-141, miR-134, miR-155, miR-346, miR-422a, miR-519a, miR-648, miR-662
Zhang et al.[141]	Epithelial ovarian cancer cell lines vs. HOSE cell line	35	Upregulated: miR-26b, miR-182, miR-103, miR-26a. Downregulated: let-7d, miR-410, miR-134, miR-100, miR-432, miR-127, miR-99a, miR-222, miR-154*
Nam et al.[64]	Twenty serous ovarian cancer vs. eight normal ovarian tissues; microarray platform, Northern blot	23	Upregulated: miR-21, miR-200a, miR-200b, miR-200c, miR-20a, miR-23a, miR-23b, miR-27a, miR-141, miR-16. Downregulated: miR-145, miR-125a, miR-125b, miR-100, miR-99a, miR-26a, miR-10b, miR-143, miR-214, miR-7b, miR-29a, miR-199-AS
Wyman et al.[41]	Thirty-three cancer tissue samples vs. HOSE cell line; massively parallel pyrosequencing technology	121	Upregulated: miR-126*, miR-142-3p, miR-195, miR-200a, miR-200b, 200c, miR- 338-3p, miR-378, miR-182. Downregulated: miR-100, miR-127-3p, miR-210, miR-22, miR-222, miR-382, miR-409-5p, miR-485-5p, miR-493. Novel microRNAs: miR-2114, miR-449c, miR-2115, miR-2116, miR-2117, miR-548q
Resnick et al.[48]	Twenty-eight sera from ovarian cancer patients vs. 15 healthy controls; microarray platform, quantitive RT-PCR	23	Upregulated: miR-21, miR-29a, miR-92, miR-93, miR-126. Downregulated: miR-127
Sorrentino et al.[90]	Drug-resistant vs. wild type cancer cell lines; microarray platform, quantitive RT-PCR, Northern blot	34	Upregulated: 125b. Downregulated: miR-335, miR-130a

Table I. Continued

Authors	Material and methods	*n*	MicroRNAs altered in the study The most significant expression alteration
Lee et al.[89]	Thirty-three high grade serous carcinoma vs. three normal fallopian tube samples	29	Upregulated: miR-200c, miR-141, miR-106a,b, miR-17-5p, miR-107, miR-20a,b, miR-181b, miR-146b, miR-103, miR-205, miR-182, miR-203, miR-15a, miR-422a,b, miR-21, miR-363. Downregulated: miR-145, miR-143, miR-34c, miR-195, miR-29c, miR-125b, let-7b,c, miR-126
	Thirty-three high grade serous carcinoma vs. two low grade serous carcinoma samples	9	Upregulated: 0. Downregulated: miR-34a,b,c, miR-449, miR-509, miR-508, miR-92, miR-514, miR-29a,c, let-7b, miR-10a
Eitan et al.[85]	Nineteen stage I vs. 38 stage III serous and endometrioid carcinoma samples	18	Upregulated in stage I: miR-423-3p, miR-130a, miR-146b-5p, miR-193a-3p, miR-193a-5p, miR-491-5p, miR-23b, miR-125a-3p, miR-125-5p, miR-451. Upregulated in stage III: miR-200a, miR-200b, miR-34a, miR-513a-5p, miR-509-3p, miR-509-3-5p, miR-574-5p, miR-449b
	Twenty-five platinum-sensitive vs. 12 platinum-resistant samples	7	Upregulated in platinum-resistant: miR-27a, miR-23a, miR-30c, let-7g, miR-199a. Upregulated in platinum-sensitive: miR-378, miR-625

year. In only 19% of cases, ovarian cancer is diagnosed in the early stage of clinical progression and approximately 70% of patients are in advanced stages of the disease at the moment of diagnosis. Due to lack of an effective screening method, asymptomatic initial disease phase and the resistance to chemotherapy regimes that occurs in 20% of patients, ovarian cancer is associated with poor prognosis. Despite an extensive number of studies concerned with ovarian cancer, the survival rates have not substantially improved over the last 30 years.[62]

Most of the studies that investigated functions of microRNAs in gynecological malignancies focused on ovarian cancer. However, results presented by different authors are not consistent and ovarian cancer microRNA signatures observed in various studies differ significantly. Alterations of only a limited number of microRNAs have been repeatedly reported in more than one study (Table I).

Among them, microRNAs belonging to miR-200 family, miR-200a, miR-200b, and miR-200c, were most consistently reported upregulated. Overexpression of those microRNAs was found in at least five independent studies suggesting their oncogenic function.[41,63–66] Bendoiraite et al. observed a correlation between an increased miR-200 family expression and a downregulation of Zn-finger transcriptional repressors ZEB1 and ZEB2, which inhibit transcription of E-cadherin in ovarian cancer. Based on their results, authors speculated that an increased expression of E-cadherin combined with upregulation of miR-200 family may support a model of epithelial cancer carcinogensis that consists in a

Table II. Summary of the MicroRNAs Expression Profiling Studies in Cervical and Endometrial Cancers and Leiomyoma

Authors	Material and methods	*n*	MicroRNAs altered in the study The most significant expression alteration
Endometrial cancer			
Boren et al.[94]	Thirty-seven cancer and 4 atypical hyperplasia vs. 20 normal endometrium tissue samples; microarray platform, quantitive RT-PCR	13	Upregulated: miR-185, miR-106a, miR-181, miR-210, miR-423, miR-107, miR-103, let-7c. Downregulated: let-7i, miR-221, miR-193, miR-152, miR-30c
Chung et al.[95]	Thirty-eight cancer tissue samples vs. 26 normal endometrium; quantitive RT-PCR	30	Upregulated: miR-205, miR-182, miR-325, miR-183a, miR-203, miR-210, miR-95
Wu et al.[96]	Ten endometroid type tissue samples vs. 10 normal endometrium; microarray platform	17	Upregulated: miR-205, miR-449, miR-429. Downregulated: miR-204, miR-99b, miR-193b
Hiroki et al.[97]	Twenty-one serous type tissue samples vs. seven normal endometrium	120	Upregulated: miR-205. Downregulated: miR-101, miR-10b*, miR-133b, miR-152, miR-29b, miR-34b, miR-411,
Cervical cancer			
Lui et al.[114]	Six cancer cell lines and five normal cervical samples; cloning-based technique, Northern blot	6	Upregulated: miR-21. Downregulated: let-7b, let-7c, miR-23b, miR-196b, miR-143
Wang et al.[117]	$HPV16^+$, $HPV18^+$, HPV-negative cervical cancer cell lines, cancer-derived cell lines; cervical cancer vs. age-matched normal cervix samples; microarray platform, Northern blot	33	Upregulated: miR-15a, miR-223, miR-146a. Downregulated: miR-143, miR-145, miR-218, miR-424
Martinez et al.[118]	$HPV16^+$, $HPV18^+$, HPV-negative cervical cancer lines and cervical cancer tissue samples vs. normal cervix samples; microarray platform, Northern blot, quantitive RT-PCR	27	Upregulated: miR-182, miR-183, miR-210. Downregulated: miR-143, miR-145, miR-126, miR-195, miR-218, miR-368, miR-497
Lee et al.[123]	Ten cancer tissue samples vs. ten normal cervix controls quantitive RT-PCR	70	Upregulated: miR-199a, miR-199s, miR-9, miR-199a*, miR-199b, miR-145, miR-133a, miR-133b, miR-214, miR-127. Downregulated: miR-149, miR-203
Leiomyoma			
Marsh et al.[130]	Fifteen leiomyoma vs. matched normal myometrium tissue samples; microarray platform, quantitive RT-PCR	46	Upregulated: miR-542-3p, miR-377, miR-582, miR-21, miR-125b, miR-323, miR-34a. Downregulated: miR-542-5p, miR-642, miR-150, miR-203, miR-139

Table II. Continued

Authors	Material and methods	*n*	MicroRNAs altered in the study The most significant expression alteration
Wang et al.[131]	Fifty-five leiomyoma vs. matched normal myometrium tissue samples; microarray platform; semiquantitive RT-PCR	45	Upregulated: miR-23b, miR-21, miR-30a, miR-145, miR-27a, let-7. Family downregulated: miR-144, miR-29b, miR-32, miR-197, miR-220, miR-212, miR-292-3p
Pan et al.[137]	Seven leiomyoma and seven matched myometrium tissue samples, isolated smooth muscle cells, spontaneously transformed smooth muscle cells and leiomyosarcoma cell line (SK-LMS-1)	91	miR-20a, miR-21, miR-26a, miR-18a, miR-206, miR-181a, and miR-142-5p

mesothelial-to-epithelial transition (MET).[66] Interestingly, different results were obtained by Cochrane et al., who found very low levels of miR-200c in poorly differentiated ovarian cancer cells in comparison to well differentiated cells. In addition, downregulation or absence of miR-200c positively correlated with increased expression of ZEB1, a molecule responsible for epithelial to mesenchymal transition (EMT) in cancer cells. Moreover, the study demonstrated that restoration of miR-200c expression resulted in reduction of invasiveness and migration capability as well as increased sensitivity to microtubule targeting cytotoxic agents.[67] Cochrane's study seems to be confirmed by results obtained by Hu et al., who found a significant correlation between low miR-200a expression and a recurrence as well as poor overall survival of ovarian cancer patients.[68] Given such inconsistent data, which of the proposed mechanisms, MET or EMT, is more probable to be involved in ovarian cancer pathogenesis remains to be elucidated.

Members of let-7 family of putative onco-supressors and miR-125b are microRNAs, which were invariably reported downregulated in ovarian cancer compared to normal ovarian epithelium.[63–65,69–74] Additionally, decrease in let-7 expression was associated with higher histological grade, resistance to chemotherapy, and shorter progression-free survival.[65,75]

Among other microRNAs reported to be differentially expressed in ovarian cancer, miR-145 was found downregulated in two independent studies and miR-126 was described to be overexpressed by another two research groups.[41,48,63] Other microRNAs, which were found altered in more than one study, include miR-21, miR-214, miR-199a, miR-99a, miR-26a, miR-10b, and miR-100. However, expression alterations of those microRNAs differed depending on the experimental design. Yang et al. observed upregulation of miR-214 and miR-199a, whereas downregulation of those particular microRNAs was found by Iorio et al. and Nam et al.[63–65] Overexpression of miR-199a was also present in the Dahiya et al. study.[76] In addition, authors of the latter study observed upregulation of miR-99a and miR-100, whereas Nam et al. and Yang et al. reported decreased expression of those microRNAs.[64,65] Downregulation of miR-100 was also reported by Wyman et al.[41] The same study reported a significant overexpression of miR-26a and miR-10b, which downregulation was described by Nam et al.

MiR-21 is considered to have pro-oncogenic abilities as its overexpression was described in several solid tumors and it was proven to inhibit PTEN and Pdcd4, two well known onco-suppressors.[15,70,77–82] Upregulation of miR-21 in ovarian cancer was reported by Resnick

et al. and Nam et al.; however, in the study performed by Dahiya et al., its expression was decreased in comparison to human ovarian surface immortalized epithelial cell line (HOSE).[48,64,76] Similar discrepancies refer to alteration of miR-15a and miR-16 observed in ovarian cancer. Decreased expression of those microRNAs was reported by Bhattacharya et al. and correlated with increased levels of Bmi-1, a protein involved in ovarian cancer proliferation and clonal growth. A direct downregulation of Bmi-1 induced by enhanced expression of miR-15a and miR-16 was also demonstrated in that study.[83] These results are, however, contrary to data acquired by other investigators, including earlier studies by Wyman et al. and Nam et al., who found miR-15a and miR-16 to be significantly upregulated in ovarian cancer tissues.[41,64] Inconsistencies existing between the results obtained in ovarian cancer studies could occur due to the differences in the study populations and methodologies used, especially the choice of a control might have a significant impact on final results. Whole normal ovaries, which are not considered fair control tissues for ovarian cancer, were used in some of the studies, whereas a number of authors utilized HOSE cell lines.[63–65,76] Nevertheless, despite methodological differences, the existence of significant discrepancies in expression profiles of certain microRNAs observed by various authors indicates the need of further and more in-depth research that would elucidate those equivocal results.

Many studies attempted to reveal correlations between microRNA signatures and clinicopathologic characteristics of ovarian malignancy. Iorio et al. observed that microRNA signatures enabled distinction between various histological subtypes of ovarian cancer.[63] Let-7 and miR-199a downregulation and increased expression of miR-200a, miR-200b, miR-200c, miR-141, miR-180a, miR-93, miR-429, and miR-29b were found to be associated with shortened disease-free survival in a number of studies.[64,65,84] One of the research groups reported five other microRNAs, miR-23a, miR-27a, miR-449b, miR-21, and miR-24–2*,to be associated with poor prognosis as well. In that study, miR-27a was found to identify individuals with progression during first-line chemotherapy and extremely short survival.[85] Overexpression of miR-214, miR-200a, and miR-199a correlated with late clinical stage and poor differentiation of the tumor as reported by other research group.[74] MicroRNA profiling performed by Laios et al. revealed differences in miR-9 and miR- 223 expression between recurrent and primary tumors. Such observation might suggest that some microRNAs are responsible exclusively for initiation of tumorigenesis, and others take part in spread and reoccurrence of malignancy.[86] Interestingly, Guo et al. observed a downregulation of miR-9 in primary ovarian cancers and demonstrated nuclear factor κB1 (NFκB1) to be a direct target of miR-9. NFκB1 can act as tumor promoter; thus, its inhibition may consist of one of the mechanisms involved in suppression of cell proliferation induced by miR-9 overexpression.[87]

Lee et al. compared microRNA signatures in ovarian cancer with respect to BRCA1/BRCA2 status and did not find any significant differences, which is quite interesting given that longer survival and better outcome were attributed to BRCA2 carrier status.[88,89] However, the authors observed significant microRNAs alterations when cancerous tissues were compared to tubal epithelium used as a control, and when comparison between high- and low-grade tumors was performed (Table I). Additionally, lower levels of miR-34c and miR-422b in high-grade tumors were associated with shorter disease-specific survival.[89] An interesting research performed recently by Resnick et al. demonstrated feasibility of small RNAs identification in sera of ovarian cancer patients and revealed expression alterations of 23 microRNAs, among which miR-21, miR-29a, miR-92, miR-93, and miR-126 were most significantly upregulated.[48] The authors were not able to, however, demonstrate any correlations between microRNAs alterations and clinicopathologic characteristics.

MicroRNA signatures were also investigated in ovarian cancers resistant to chemotherapy. Yang et al. reported overexpression of miR-214 to increase cell viability and

induce resistance against cisplatin, through downregulation of PTEN expression and activation of Akt pathway.[74] Tumors resistant to standard chemotherapy were characterized by decreased expression of miR-335 and miR-130a in the study performed by Sorrentino et al.[90] Boren et al. demonstrated a significant association between altered expression of 27 microRNAs in OVCA cancer cell lines and response to 6 commonly used chemotherapeutics. Importantly, increased expression of 7 microRNAs, miR-213, miR-181a, miR-181b, miR-99b, miR-514, miR-518c-AS, and miR-520f, correlated with resistance to more than 1 chemotherapy regime.[91] Neither miR-335 nor miR-130a, indicated by Sorrentino et al., were, however, altered in the Boren study. Eitan et al. identified five other microRNAs that were associated with platinum-resistant tumors (Table II).

B. MicroRNA Studies in Endometrial Cancer

According to CDC statistics, endometrial cancer is the fourth most common cancer in the female population. In most cases, it is diagnosed in early stages of clinical progression which enables radical treatment and good prognosis. However, an advanced stage at diagnosis or a recurrent disease significantly worsens outcomes and increases mortality.

MicroRNAs seem to be promising in unraveling complicated biomolecular pathways of endometrial carcinogenesis, as some of the genes, in which roles have been implicated in that malignancy, were proven to be regulated by microRNAs.[92,93]

Most of the microRNA profiling studies performed to date focused on endometroid subtype of endometrial carcinoma and revealed a number of differentially expressed microRNAs (Table II).

Among them, five were reported differentially expressed by at least two independent studies. MiR-103, miR-106a, miR-107, and miR-210 were upregulated in the studies performed by Boren et al. and Chung et al., and miR-205 was found highly overexpressed by another 3 research groups.[94–97] Not only was miR-205 the most upregulated in those studies, but it also positively correlated with the advanced clinical stage and the degree of myometrial invasion, as reported by Chung et al.[95] In addition, 5 other microRNAs, miR-182, miR-183, miR-200a, miR-34a, and miR-95, significantly overexpressed in that study were strongly associated with tumor progression and lymph nodes metastases.[95] Conversely, an analysis performed by Boren et al. did not reveal any correlations between altered microRNA expression profiles and clinicopathologic features.[94] Interestingly, of the 30 microRNAs which were altered in Chung's study, 20 were earlier reported to be up or downregulated in other malignancies, including leukemia, ovarian, cervical, pancreatic, and bladder cancers.[98–101] The group of those altered microRNAs enclosed miR-200a and miR-200c, in which overexpression was reported by several ovarian cancer studies, suggested a possibility of a common mechanism involved in ovarian and endometrial carcinogenesis. A recent study by Huang et al. revealed a reciprocal association between downregulation of miR-129-2 and sex determining region Y (SRY)-box 4 (SOX4) expression in endometrioid endometrial cancer samples. The same authors demonstrated also that restoration of miR-129-2 induced a decrease in SOX4 expression and resulted in diminished cells proliferation.[102]

Serous type comprises approximately 10% of endometrial cancer cases and is characterized by a more aggressive course, more frequent lymph node involvement, and worse prognosis compared to endometroid histology. Altered expression of 120 microRNAs was demonstrated in 21 patients diagnosed with serous endometrial carcinoma in a recent study performed by Hiroki et al.[97] The largest increase in expression was attributed to miR-205, in which the upregulation was also reported in earlier studies.[94,95] The authors found that decreased expression of miR-10b*, miR-29b, and miR-455-5p was associated with vascular invasion, and that downregulation of miR-101, miR-10b*, miR-139-5p, miR-152, miR-29b,

and miR-455-5p correlated with poor overall survival. In addition, decreased expression of miR-101 and miR-152 was found to consist of an independent risk factor for disease-free survival, and downregulation of miR-152 alone was an independent risk factor for overall survival. Interestingly, restoration of those microRNAs in serous endometrial cancer cell line by transfection led to diminished cells proliferation. Moreover, the study revealed that the downregulation of miR-101 was correlated with strong positive immunoreactivity of cyclooxygenase-2, in which the increased expression was earlier demonstrated in endometrial cancer and associated with worse prognosis.[97,103,104]

Endometrial cancer is well established enough to be an estrogen-dependent malignancy. In this context, possible estrogen-dependent microRNA regulation is worth considering. MicroRNAs were proved to be regulated by estradiol (E_2) and estrogen receptor α and β agonists (ERα, ERβ) in animal studies and also in human breast cancer, endometrial, and leiomyoma cell lines.[105,106] A cohort of 38 microRNAs differentially expressed in MCF-7 cell line treated with E_2 included several microRNAs, in which alteration was documented in endometrial cancer; for instance, miR-200a, miR-200b, miR-106b, miR-182, and let7-c were upregulated, whereas miR-200c was downregulated.[107] In addition, inhibition of miR-206 expression was observed in MCF-7 line treated with ERα agonist, in the study performed by Adams et al.[108] Toloubeydokhti et al. observed expression alterations of miR-17-5p, miR-23a, miR-23b, and miR-542-3p in epithelial and stromal endometrial cell lines exposed to E_2, medroxyprogesterone and their respective antagonists.[109] Importantly, 4 microRNAs, miR-22, miR-221, miR-222, and miR-206, were also proven to decrease ERα expression in MCF-7 cells, which might suggest the mutual relation between microRNAs and ERs expression in tissues.[107] To date, no data are available on relationships between microRNAs and estrogen response in endometrial cancer; thus, this concept consists of an interesting research subject.

C. MicroRNA Studies in Cervical Cancer

More than 400,000 new cases of cervical cancer that lead to approximately 270,000 deaths are diagnosed every year worldwide.[110] Cervical cancer etiology is strongly linked to human papilloma virus (HPV) infection, and the involvement of E6 and E7 HPV proteins has been well documented in its pathogenesis. Nevertheless, the exact pathway leading from the infection to tumorigenesis has not been revealed so far.[111,112] High efficacy of the screening has significantly decreased the rates of cervical cancer diagnoses over the past 20 years; however, in underdeveloped countries, it is still one of the leading causes of death in female population. It is also responsible for the highest number of the years of life lost from cancer in the developing world.[110,113]

Emerging role of microRNAs in cervical carcinoma has been postulated by an enlarging number of studies (Table II). Using a cloning-based technique for microRNAs profiling, Lui et al. documented down-modulation of let-7b, let-7c, miR-23b, miR-196b, miR-143, and increase of miR-21 expression.[114] MiR-21 upregulation was observed in many solid tumors and its function was linked to inhibition of several tumor suppressor genes involved in regulation of cells proliferation and apoptosis.[15,70,77–82] Interestingly, Yao et al. found that loss of miR-21 expression in HeLa cells resulted in diminished proliferation and increased expression of Pdcd4, a well-known tumor suppressor. Contrary results were obtained by Cheng et al., who demonstrated that the knockdown of miR-21 induced cell growth in HeLa cell line.[115,116] Given such equivocal results, further studies are necessary to unravel complicated pathways related to miR-21. Downregulation of miR-143 observed by Lui et al. was confirmed by other research groups, including Wang et al. and Martinez et al., suggesting that particular microRNA to play an important role in cervical

carcinogenesis.[114,117,118] However, miR-143 does not seem to be specific for this type of cancer, as significant decrease in its expression was observed also in other tumors, e.g. colorectal, breast, and lymphoid cancers.[70,119,120] Significant alterations of microRNAs expression were observed both in cervical cancer and HPV-induced premalignant lesions by Wang et al. Interestingly, tissues sampled from cervical cancer, but not the HPV-induced premalignant lesions or cancer-derived cell lines, were characterized by significantly increased expression of miR-146a. The authors also proved that transfection of miR-146a into HPV-positive cervical cancer cell line significantly increased cell doubling time and promoted cell proliferation. The absence of miR-146a in premalignant lesions and homogenous cancer cell cultures suggests that its presence is more important for tumor progression than for initiation of carcinogenesis or that it derives from a cell population other than epithelial cervical cancer cells.[117] Martinez et al. studied microRNA expression profiles in HPV-16 positive, HPV-18 positive, HPV-negative cervical cancer lines and in cervical cancer tissue samples. Twenty-seven microRNAs (Table II), including those reported by Lui et al. and Wang et al., were aberrantly expressed in HPV-16 positive cervical cancer cell lines. In addition, miR-143 and miR-145 were equally down-modulated in all cervical cancer cell lines, including HPV-negative C-33A cells, what according to the authors could implicate that their role in cervical tumorigenesis was independent of HPV infection. Conversely, miR-218 was the sole microRNA to be downregulated in HPV-16 and HPV-18 positive cell lines in comparison to normal cervical tissue and C-33A cells. Its expression was also decreased in cervical cancer and CIN III tissues. Thus, the authors suggested its potential role as a specific cellular target of high-risk human papilloma viruses. Moreover, functional experiments performed by the same group revealed association between decreased expression of miR-218 and E6 protein of high-risk HPVs level. They also demonstrated a capability of miR-218 to decrease expression of laminin 5 β3, which was proven to promote tumorigenesis in human keratinocytes, augment cell migration in SCID mice, and serve the HPVs as a receptor during invasion of cervical epithelium basal cells.[118,121,122] Authors concluded that E6 modulated down-regulation of miR-218 with the subsequent overexpression of LAMB3 could facilitate HPV infection of cervical tissues and thus promote carcinogenesis.[118] It is worth mentioning that upregulation of miR-218 was also reported in the study performed by Wang et al.[117] Among 70 microRNAs, in which alterations were found by Lee et al., miR-199a, miR-127, and miR-214 were most significantly upregulated. Further experiments performed by that research group revealed that miR-199a inhibition by anti-miR-199a oligonucleotides resulted in depressed cell growth in vitro and the analysis of clinicopathologic features demonstrated a positive correlation between overexpression of miR-127 and a positive lymph nodes status.[123] Conversely, to the results obtained by Lee et al., Yang et al. reported downregulation of miR-214 in cervical cancer and showed its ability to inhibit HeLa cells proliferation by targeting MEK3 and JNK1 transcripts.[124]

The experimental study performed by Wang et al. demonstrated that E6 induced de-stabilization of p53 protein, downregulated the expression of miR-34a, and led to increased cell proliferation in pre-malignant HPV infected and cervical cancer tissues as well as in cervical cancer cell lines. To our knowledge, this is the first study to report viral regulation of tumor suppressor microRNA expression.[125]

D. MicroRNA Studies in Leiomyomas

Uterine fibroids are the most common benign tumors diagnosed in the female population. The prevalence of that pathology ranges from 70 to 75% in patients of Caucasian origin and is higher, of approximately 80%, in African-American women. Even though in many cases leiomyomas are asymptomatic, they still constitute common causes of hospitalizations and

hysterectomies due to gynecological pathology.[126] Etiology of these sex steroid-dependent tumors is largely unknown despite their common incidence rate. Genetic alterations, including nonrandom chromosomal anomalies and gene mutations, have been discovered in leiomyoma tissues. Additionally, microarray studies have revealed an altered expression of many genes involved in cell proliferation, differentiation, and apoptosis in uterine fibromas (Table II).[127,128] MicroRNAs' role has also been investigated in the pathogenesis of leiomyomas. An analysis performed by Luo et al. revealed that 27 microRNAs were deregulated in at least 2 of the currently published profiling studies, and miR-21 was reported altered in all three of them.[129] The study by Marsh et al. evaluated expression of 454 microRNAs and revealed up-modulation of 19 and downregulation of 27 microRNAs, with miR-21, miR-125b, miR-323, and miR-34a being most significantly increased and miR-139 most significantly decreased.[130] Highly increased mir-21 expression was also found by Wang et al.[131] Significant function of miR-21 in the pathogenesis of leiomyomas was recently demonstrated by Pan et al. who found it to be hormonally regulated and to influence critical processes of leiomyoma development through interaction with TGF- β signaling pathway.[132]

Other microRNAs which were significantly altered in the Wang et al. study included miR-197, miR-23b, miR-29b, and let-7 family. In addition, the authors observed differences in expression patterns of miR-23a/b, let-7s, miR-124, miR-197, miR-411, and miR-412 between black and white women. Interestingly, the let-7 family, which has been reported to be downregulated in most malignant neoplasm, was found to be highly upregulated in that study.[70,131,133] The authors also observed that high expression levels of let-7 characterized small rather than large fibroids. Further experiments performed by Wang et al. proved a negative regulation of high mobility group A2 protein (HMGA2) expression by let-7. Overexpression of HMGA2 protein has been earlier observed in large uterine fibroids, which could suggest its role in tumor growth intensification.[134] Data obtained by Wang et al. provided, therefore, a new insight into the pathogenesis and growth conditions of leiomyomas. Those observations were further confirmed by Peng et al. whose in vivo and in vitro studies demonstrated additional support for the thesis that dysregulation of let-7s induced repression of HMGA2 could be a specific molecular mechanism involved in promotion of leiomyoma growth.[135] Interestingly, the same group of authors found increased levels of let-7c and let-7d in senescent fibroids with a low Ki-67 index, which could suggest an important role for let-7 in eliciting cellular senescence in leiomyomas.[136] Concordant results were obtained by Pan et al. who studied expression profiles of 385 microRNAs in paired leiomyoma and myometrium tissues, isolated smooth muscle cells, spontaneously transformed smooth muscle cells, and leiomyosarcoma cell line. Ninety-one microRNAs were found to be downregulated in leiomyomas compared to normal endometrium, with greatest alteration attributed to miR-20a, miR-21, miR-26a, miR-18a, miR-206, miR-181a, and miR-142-5p expression. Moreover, even greater decline in microRNAs levels was noticed in isolated as well as in spontaneously transformed cells and sarcoma cell lines. Furthermore, treatment of cells isolated from leiomyoma and myometrium with ovarian steroids and their inhibitors resulted in additional alterations in microRNAs levels, which depended on the pharmaceutical used as well as on the type of the cell line subjected to its influence.[137]

3. MECHANISMS RESPONSIBLE FOR MICRORNA EXPRESSION ALTERATIONS IN GYNECOLOGICAL TUMORS

Several defined and putative mechanisms responsible for microRNA expression alterations in tumors have been identified. They include aberrations in microRNA encoding sequences,

dysregulation of microRNA biogenesis, and mutations in sequences responsible for interactions between microRNA and mRNA.[138–140] At least half the microRNA encoding genes are located in the chromosomal loci that are unstable in cancer and frequently suffer from genomic alterations, such as deletions, insertions, inversions, and translocations.[138] Examples of such microRNAs include miR-142 located at the breakpoint junction of a t(8,17) translocation, miR-15a and miR-16a embedded within chromosome 13q14, a region commonly deleted in B cell chronic lymphocytic leukemia or miR-34a encoded by the Ch1p36 region, which was commonly deleted in ovarian cancer, as reported by Kuo et al.[139–142] Furthermore, Zhang et al. suggested that DNA copy number alterations could contribute to microRNA deregulation observed in malignancies. Such correlation was confirmed for a putative oncogenic miR-182, in which chromosomal locus chr 7_129-130 was amplified in 28.9% of ovarian cancers and correlated with overexpression of that microRNA as well as for a known tumors suppressor miR-15a, which was downregulated in 23.9% of ovarian cancers. Reduced expression of miR-15a positively correlated with deletion of locus chr 13_49-50, which contains miR-15a encoding sequence.[141]

The role of point mutations in pri-microRNA encoding sequences was also postulated by some authors; however, recent studies did not reveal the presence of such genomic aberrations in ovarian cancer-derived cell lines.[143] A single nucleotide polymorphism contribution to microRNA expression dysregulation was also indicated. As demonstrated by Shen et al., G to C polymorphism (rs 2910164) located within the sequence encoding for miR-146a precursor, which targets BRCA1 and BRCA2 messenger RNAs, was associated with higher expression of mature miR-146 and younger age of diagnoses in familial breast and ovarian cancers.[144] Epigenetic regulation of microRNA encoding sequences constitutes another possible mechanism of their dysregulation in human cancer. Two mechanisms of such regulation are currently being extensively investigated, one based on DNA hypermethylation and the other consists in histone modifications leading to chromatine remodeling.[145] Many studies proved that abnormal DNA methylation patterns exist in the genome of cancer cells. Hypermethylation of CpG islands of the onco-suppresors promoter region, which eventually leads to their silencing, has been the most extensively evaluated and suggested to induce dysregulation of several genes in various malignancies, including $p16^{ink4A}$ gene in lung cancer, BRCA1 gene in breast cancer, and VHL gene in kidney cancer.[146–148] Recent studies seem to prove that dysregulation of epigenetic mechanisms could alter microRNAs expression in cancer. The study conducted by Saito et al. demonstrated that treatment of bladder cancer cells with DNA demethylating agent and histone deacetylase inhibitor was responsible for the upregulation of approximately 5% of human microRNAs with miR-127, in which coding sequence is embedded in a CpG island being most significantly induced.[149] Lu et al. presented concordant results. In their study, decreased methylation of a sequence encoding let-7a-3 correlated with up-modulation of this microRNA, high expression of IGF-II and IGFBP-3, and a poor prognosis in ovarian cancer.[150] Iorio et al. suggested that overexpression of some microRNAs, including miR-21 and miR-203, observed in epithelial ovarian cancer, could be a result of DNA hypomethylation.[63] A recent study by Lehmann et al. demonstrated correlation between down-modulation of mir-9-1 and its sequence hypermethylation in breast cancer tissues.[151] Similar results were obtained by Huang et al. who reported an association between loss in miR-129-2 expression and hypermethylation of the miR-129-2 CpG island in endometrial cancer tissues, which correlated with poor overall survival. Furthermore, a restoration of miR-129-2 occurred in endometrial cancer cells after treatment with histone acetylation inducing and DNA demethylating agents.[102]

Better understanding of epigenetically regulated microRNAs expression modulation may be of importance with regard to the development of new treatment strategies, all the

more since, recently, three substances targeting epigenetic changes have been approved by FDA for treatment of myelodysplastic syndrome and cutaneous T-cell lymphoma.[152]

Dysregulation of microRNA expression caused by improper function of microRNA processing apparatus have been proven in a number of studies. Thomson et al. demonstrated that global downregulation of microRNAs expression observed in primary malignant tumors was connected with abnormal microRNA processing at the stage of Drosha activity.[153] Similar results were obtained by Muralidhar et al. who observed a correlation between increased Drosha expression and up-modulation of oncogenic microRNAs in cervical cancer tissue.[154] Scotto et al. proved the connection between Drosha overexpression and the genomic gain of chromosome 5p, and suggested it to be an important mechanism of cervical cancer progression.[155] Flavin et al. observed increased expression of Dicer 1 and eIF6 protein in ovarian cancer which correlated with shorter remission interval, and suggested the possibility of using Dicer, Drosha, and eIF6 expression profiles as predictors of the efficacy and toxicity of siRNA-based therapy.[156] Contrary results were obtained independently by Merritt et al. and Pampalakis et al. who found Dicer to be downregulated in ovarian cancer and to correlate with advanced disease stages.[157,158] Other authors also confirmed the relationship between deterioration of microRNA maturation and tumor development, lymph node metastases, advanced clinical stage and overall worse prognosis in breast, lung, and prostate cancers.[159–162]

Another possible mechanism leading to microRNAs alterations in cancer has been recently reported by Dahl et al., who demonstrated epidermal growth factor treatment to induce transcriptional repression of miR-125a through the transcription factor PEA3 belonging to ETS family in ovarian cancer cell lines.[163]

4. PERSPECTIVES ON MICRORNAS UTILIZATION AS DIAGNOSTIC AND THERAPEUTIC TOOLS

MicroRNAs profiling studies have provided interesting data suggesting that altered expression of specific microRNAs in cancer tissues may correlate with patient survival rates and risk of recurrence, and could be, therefore, used for predicting disease outcome.[64,70] Correlation between the altered expression of certain microRNAs and tumor progression, chemoresponse, and patients' survival was reported in ovarian and endometrial cancers as well as in other malignancies, including colorectal adenocarcinomas, chronic lymphocytic leukemia, lung, breast, and pancreatic cancers.[64,65,70,95,164–168] There are also reports suggesting microRNAs to alter resistance to cytotoxic therapy.[169]

More recent data suggest that microRNA populations isolated from serum or plasma could consist of an easy and less time-consuming tool for microRNA profiling compared to tissue-based methods. Serum- or plasma-based microRNA diagnostic tests could be used for cancer screening, monitoring of treatment, and sensitive tests for chemotherapy response.[46,47] The aforementioned study performed by Resnick et al. demonstrated that serum-derived microRNAs profiling was feasible and could potentially serve as an early diagnostic test, especially in patients with normal Ca-125 levels.[48] Moreover, at the 2009 American Association of Cancer Research Annual Meeting, a noninvasive serum-based diagnostic test screening for colorectal cancer was presented and is expected to be available by year 2010 (https://www.rosettagenomics.com/inner.asp?first_tier = 162).

Since it has been confirmed that particular microRNAs supervise posttranscriptional processing of hundreds of various transcripts, modification of single microRNA expression could potentially influence multiple pathways involved in carcinogenesis.[170] One of the approaches which has been extensively investigated consists in inhibition of microRNAs by

modifier molecules, such as 2-O-methyl oligorybonucleotides called antagomirs, locked nucleic acid-modified oligonucleotides, and anti-miRNA oligonucleotides.[171–173] Antagomirs effectively inhibited microRNAs in mice experimental model and in mammalian cell culture.[174,175] Tsuda et al. proved that inhibitors and activators of certain microRNAs were capable of decreasing cell proliferation in pancreatic cancer cell line.[176] A more recent study by the same research group presented synthetic microRNAs and corresponding duplex/small temporal RNAs, to effectively inhibit ovarian cancer cells proliferation through targeting glioma-associated antigen-1.[177] An interesting approach to inhibition of microRNAs activity, ''miRNA sponge,'' has been presented by Ebert et al. and consisted in competitive binding of target microRNAs to artificial genes containing multiple tandem-binding sites for those microRNAs.[178] Such strategy was recently demonstrated to be effective in vivo in breast cancer.[179]

A fairly new approach to microRNA-based therapy employs molecular manipulation in order to restore expression of certain microRNAs by target cells.[180] Such approach may turn out even more efficacious given that a large number of microRNAs is downregulated in malignant tumors and that a global decrease in microRNAs expression as well as deterioration of microRNA processing machinery was linked to the enhancement of cell transformation and tumor development.[181,182] MicroRNAs delivery seems to be more advantageous compared to the techniques based on RNA interfering systems. Basing on the fact that microRNAs are endogenous regulators, mechanisms that would attenuate their dysregulation are likely to occur in cells, decreasing the risk of the off-target effects after their therapeutic delivery. In addition, an ability to target multiple pathways by single microRNAs seems to decrease possibility of developing resistance to the treatment, as several mutations occurring at the same time would be necessary to overcome their multidirectional activities.[183] In respect, thereof, very interesting results has been recently presented by Kota et al., who demonstrated the feasibility of the adeno-associated virus vector system of miR-26a delivery in suppression of liver cancer progression without significant toxic side effects. MiR-26a has a wide spectrum of activities targeting multiple genes, including Myc and p53 network. It is abundant in healthy tissues and significantly suppressed in liver cancer cells.[183] In addition, in a large cohort of patients with hepatocellular carcinoma, its downregulation was associated with shorter overall survival and better response to interferon α therapy.[184] The results obtained by Kota et al. seem to support the thesis that the treatment based on microRNAs' potential to regulated a broad, however, not precisely specified spectrum of cellular processes, could be equally effective to systems targeting specific initiating oncogens.[183] Usefulness of microRNAs in controlling expression of transgenes in gene therapy was also postulated. A method of incorporation, certain carefully matched microRNA target sites into mRNA used for therapy, may become a way to limit its expression only to target tissues and, therefore, decrease toxicity and occurrence of side effects.[185]

Epigenetic therapy constitutes other possible targeting microRNAs in human cancers. Inhibitors of DNA methylation and histone deacetylation were proven to restore expression of tumor suppressor microRNAs in cancer cell lines, including those regulated from their own promoters, and also those located within introns of their host genes. A recent study by Saito et al. has shown intronic miR-126 to be upregulated concomitantly with its host gene EGFL7, after treatment with inhibitors of DNA methylation and histone deacetylation.[186]

Given the evidence of the microRNAs therapeutic potential their use in cancer therapy is likely just a matter of time. However, the implementation of microRNAs-targeted treatment has to be preceded by functional studies that would determine the exact roles of individual microRNAs in particular diseases. Safe and effective technology of microRNAs delivery needs to be also developed and verified by careful assessment of possible off-target effects that could result in severe toxic complications.

5. CONCLUSIONS

Altogether, the literature data suggests that microRNAs play a pivotal role in the pathogenesis of both malignant and benign tumors of the female reproductive tract. However, most of the studies describing microRNAs role in gynecological cancer tend to be phenomenological and do not reveal the exact cause of their alterations in cancer nor prove a direct influence on triggering cancerous process. Given that microRNAs are also overexpressed in many other pathological states, including inflammatory and autoimmunological disease, no effort should be spared in order to reveal whether altered microRNAs expression is a reason for carcinogenesis onset or simply a phenomena that accompanies uncontrolled cancerous cell proliferation.

Recent studies indicate that these tiny molecules are promising tools in diagnostics and gene therapy; however, the exact actions of individual microRNAs in physiological and pathological states still wait to be elucidated. Therefore, 16 years after microRNAs discovery, far too many questions still remain unanswered in order to draw final conclusions on their role and true potential for utilization in the clinical management of gynecological tumors.

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