microRNAs, angiogenesis and fibrinolysis in gynecological pathology

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**ABSTRACT**

miRNAs function as important regulators of a wide range of cellular processes, such as angiogenesis and fibrinolysis, by posttranscriptional modulation of gene expression. We present a review on the role of miRNAs, angiogenesis and fibrinolysis in benign and malignant gynecological pathologies of the female reproductive tract.

Endometriosis, defined as the implantation of endometrial tissue outside the uterine cavity. It is one of the most frequent benign gynecological diseases and it has important consequences in the quality of life and fertility of patients on their reproductive period. Similarly to tumor metastasis, the ectopic endometrium acquires the capability to adhere, proliferate and infiltrate the extracellular matrix. Neovascularization is therefore required to establish the peritoneal lesion, which will progressively grow and infiltrate the surrounding tissue. Even if many theories have been proposed in order to explain this disease, the exact pathogenic mechanism of endometriosis remains unsolved. In addition, a relationship between endometriosis and several gynecological cancers, especially ovarian cancer, has been reported. Consequently, the fact that miRNAs circulate confined within exosomes gives them stability in many biological fluids making them an important role as emerging biomarkers of patients’ outcome in terms of survival and recurrence of the disease. Recently, miRNAs have been detected in serum and plasma.

miRNAs are implicated in many important biological processes and they play an important role in cancer as regulatory molecules, acting as oncogenes (oncomiRs) or tumor suppressors. miRNAs are aberrantly expressed in different human cancer types, including endometrial, cervical and ovarian cancer, and they can potentially act as biomarkers of patients’ outcome in terms of survival and recurrence of the disease. On the other hand, the development of therapies that might block the expression or mimic the functions of miRNAs could represent new therapeutic strategies for any of the aforementioned gynecological disorders.

**microRNAs**

microRNAs (miRNAs) are small 21-22 nucleotide non-coding RNAs that regulate gene expression and play a key role in a wide range of biological processes. These small molecules bind to the 3' untranslated regions (3'-UTR) of their target mRNAs, mediating translational repression and/or mRNA degradation.

Although miRNAs were first discovered in 1993, it has been more recently when research endeavors have suggested and reinforced their role as important regulators of gene expression in most cellular processes and a broad spectrum of diseases.

Several conditions of miRNAs provide them the capability to act as ideal biomarkers to assess the presence or prognosis of several gynecological diseases. miRNAs lack of known post-processing modifications, have low complexity and are present at diverse body fluids. miRNAs can be analyzed not only in biological fluids (plasma, serum, peritoneal fluid, etc.) but also in fresh frozen tissues and in formalin-fixed paraffin-embedded tissues. As miRNA studies require low amounts of tissue or biological fluids, these molecules provide new tools for non-invasive molecular diagnosis and follow up.

Abnormal miRNA signatures are associated with several human diseases, including cancer, cardiovascular disorders and benign or malignant disorders of the human female reproductive tract. The altered miRNA pattern in women with endometriosis may promote cancer by enhancing or inactivating different oncogenic and tumor suppressor target genes. Thus, this miRNA dysregulation could give the rationale for the observed two-fold increased risk for ovarian cancer reported in this population. Therefore, unraveling the role of miRNAs in gynecological diseases, such as ovarian and endometrial cancer, might provide important tools to ascertain the potential of malignant transformation in endometriosis.

**microRNAs, angiogenesis and fibrinolysis in endometriosis**

Endometriosis is defined by the presence of endometrial glands and stroma outside the uterine cavity affecting up to half of the patients with pain and infertility and resulting in important impairment of their quality of life. To establish the endometriotic lesion ectopic endometrium has to survive outside the uterus, avoid the immunity mechanisms and, mimicking tumor metastases, attach to the peritoneum or other locations, infiltrate the extracellular matrix and create a vessel network through activation of angiogenesis. Endometriosis includes a wide range of lesions at different locations and three different entities can be differentiated: endometriotic implants on the surface of the peritoneum, ovarian cysts lined by endometrioid glands, and fibrotic masses...
comprising endometriotic, adipose and fibromuscular tissues, usually located between the rectum and the vagina (rectovaginal endometriotic nodule). Moreover, peritoneal implants can be divided in active or red lesions (highly vascularized) and inactive or black lesions (more fibrotic). Although it remains uncertain whether these three types are variants of the same pathologic process or caused by different mechanisms(20,21), anyhow it seems clear that their specific clinical features warrant an individualized analysis of their biological behavior.

Endometriosis is a benign disease that behaves in several aspects such as cancer. For instance, endometriotic tissue metastasizes outside the uterine cavity and infiltrates the surrounding tissue progressing along the time if no therapy is adopted. This disease is classified as a tumor-like lesion by the World Health Organization Histologic Classification of Ovarian Tumors(22). In 1925, Sampson was the first to report a case of a suspected malignant transformation in endometriosis(23). Since then, several studies have focused on the relationship between endometriosis and gynecological cancers, especially ovarian cancer(17,24).

Endometriosis is believed to be a multifactorial and polygenic disease(23,35) and emerging data provide evidence that dysregulation of miRNA expression may be involved(6,10-13,20,24,26-28,30-36). miRNAs appear to be potent regulators of gene expression in endometriosis, raising the prospect of using miRNAs as biomarkers and therapeutic tools of the disease(38).

It has been reported that angiogenesis may play an important role in the pathogenesis of endometriosis. Similarly to tumor metastases, endometriotic implants require neovascularization to proliferate, invade the extracellular matrix and establish an endometriotic lesion(19). Vascular endothelial growth factor (VEGF) represents one of the most potent angiogenic factors(37,38). Several studies have reported an increase in VEGF-A levels in endometriosis and it has been suggested that VEGF plays an important role in the progression of the disease(35,37,38). Thrombospondin-1 (TSP-1) is an inhibitor of angiogenesis and it has been reported that alterations in TSP-1 expression may be involved(6,32,34). Specifically, Burney et al. demonstrated a different expression of two miRNA families, miR-9 and miR-34, in the eutopic endometrium of women with endometriosis compared to control endometrium. One of the predicted miRNA targets of miR-9 is the anti-apoptotic Bcl2, found to be over-expressed in the eutopic endometrium of women with endometriosis(6). In relation to the miR-34 family, functional studies have concluded that both miR-34b and miR-34c act as mediators of the p53-dependent suppression of proliferation(6).

Although further studies are necessary to elucidate the role of miRNA expression in the eutopic endometrium and endometriotic tissues, miRNAs have definitely become attractive candidates in the search for novel diagnostic biomarkers to guide therapeutic interventions in endometriosis, as recently demonstrated in other miRNA-regulated diseases(35).

**Endometrial cancer, angiogenesis, fibrinolysis and microRNAs**

Endometrial cancer is one of the most frequent malignancies in the developed countries and the majority of patients are diagnosed at early stages where no metastases have yet occurred, and when the surgical therapy is often curative. In contrast, patients with advanced-stage or recurrent endometrial cancer do not respond as well to therapy and have a worse prognosis(48). Nevertheless, the pathological mechanisms responsible for cancer progression and the metastatic process in this type of cancer is one of the least studied and worst understood amongst gynecological malignancies. Gene expression profiling studies have demonstrated that many genes are dysregulated in cancer. An altered miRNAs’ expression pattern is involved in the initiation, progression, and metastatization of human cancer through the down-regulation of different proteins with oncogenic activity, thus acting as tumor suppressors, or though the down-regulation of tumor suppressors, thus acting as oncogenes (oncomiRs). miRNAs are also implicated in the process of cell adhesion and extracellular matrix
modulation and therefore might have a role in the metastatic process and cancer progression.

Recently, dysregulation of miRNA expression in endometrial adenocarcinoma has been reported by several authors. These eight reports used hybridisation array and/or RT-PCR-based methods in order to evaluate miRNA expression in endometrial cancer tissues.

The most remarkable similarity in the different studies is the up-regulation of the miR-200 family in endometrial cancer tissue compared to normal controls. The miR-200 family includes five miRNAs localized in two genomic clusters since the miR-200a/b and miR-429 are located in chromosome 1, whereas the miR-200c and miR-141 lie in chromosome 12.

Moreover, a significant overexpression of miR 205 and miR 210 has been reported in endometrial cancer. The miR-205 as well as the miR-200 family have been implicated in the epithelial-to-mesenchymal transition, tumor invasion and metastases growth. An over-expression of the miR-205 was observed in ovarian, bladder and kidney cancers. Similarly, the miR-203 was found to be significantly over-expressed in five of the eight studies so far reported on endometrial cancer and it has been reported that over-expression of this miRNA is due to hypomethylation in ovarian cancer.

The over-expression of miR 205, miR 210 and the miR 200 family suggests that these miRNAs could be selected as biomarkers for prognosis and possibly early diagnosis of endometrial cancer.

Moreover, miR-182 and miR-183 have been found to be up-regulated in four of the eight mentioned studies. The expression of the tumor-suppressor gene FOXO1 has been found to be repressed in endometrial cancer by the miR-182 and the miR-183. Similar findings have been described in ovarian cancers. Other over-expressed miRNAs in endometrial cancer are miR-135a and miR-135b. These miRNAs have been related with Wnt signalling pathway, which is known to be involved in a variety of different tumors development. On the other hand, miR-133a and miR-133b were significantly underexpressed in endometrial cancer and it has been described that they target the oncoprotein pyruvate kinase type M2 (PKM2), the oncogenic fascin 1 (FSCN1) and the proto-oncogen MET, consequently, acting like oncomiRs.

miRNA implication in angiogenesis is bidirectional, so it is possible to classify the miRNAs implicated in angiogenesis regulation under 2 categories: 1) miRNAs that target genes involved in angiogenesis, and 2) miRNAs whose expression can be modulated by proangiogenic or antiangiogenic stimuli.

In a recent study, our group has found a dysregulated expression of miRNAs related to angiogenesis and an increase in the VEGF-A levels were observed in endometrial cancer in comparison with control. The different expression of miRNAs could modulate the expression of angiogenic and antiangiogenic factors, which may play an important role in the pathogenesis of endometrial cancer.

In relation to fibrinolytic system we have observed a significant increase in plasminogen activator inhibitor-1 (PAI-1) levels was observed in endometrial cancer in comparison with the endometrial tissue from control women. Moreover, frequencies of the PAI-1 4G allele and 4G/4G genotype were found significantly more often in women with endometrial cancer than in controls. PAI-1 levels in endometrial tissue seem to be associated with PAI-1 4G/4G polymorphism. These findings suggest that the PAI-1 4G/4G genotype may be associated with the risk of endometrial cancer in a Caucasian population.

**MicroRNAs and ovarian cancer**

Ovarian cancer is the fifth leading cause of all cancer-related deaths among women. Over 80% of patients at the time of diagnosis were at an advanced stage of the disease, and the majority survived less than 5 years. Moreover, a high percentage of patients with advanced stage cancer may suffer recurrences.

miRNA expression profiling has been identified as a potential predictor for metastases and recurrence in different type of cancers. Therefore, characterization of specific biomarkers for early detection of ovarian cancer as a tumor miRNA profile could perhaps increase the survival rate.

A comparison between ovarian cancer and ovarian normal tissue identified differences in miRNA expression profile. Several research groups have identified some miRNAs that are up-regulated in ovarian cancers. Similarly, the miR-203 was found to be significantly over-expressed in five of the eight studies so far reported on endometrial cancer and it has been reported that over-expression of this miRNA is due to hypomethylation in ovarian cancer.

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-141, -200a, -200c, -200b, -205, -214) were higher in tumor-derived exosomes isolated from ovarian cancer serum compared with serum from benign ovarian disease patients\(^7\). In another study, using sera from 19 patients and 11 controls, miR-21, miR-29a, miR-126, miR-92 and miR-93 were found to be up-regulated and miR-127, miR-99b and miR-155 were down-regulated in serum obtained from patients with ovarian cancer\(^7\). Moreover, miR-21, miR-92 and miR-93 were over-expressed in 3 patients with normal CA-125 levels\(^8\), suggesting that miRNA profile may become a novel and more sensible biomarker for cancer detection.

In a recent report\(^7\), the miRNA expression profile was studied in whole blood from 24 patients with ovarian cancer and from 15 healthy controls. In this study, four miRNAs were significantly dysregulated in ovarian cancer; namely miR-30c1* , which was up-regulated, and miR-181a*, miR-342-3p and miR-450b-5p, which were down-regulated in patients.

Over-expressed miRNAs in the circulation may be a consequence of tumor growth and cell lysis or may arise from cells infiltrating the tumor.

**Future perspectives**

miRNAs have been proposed as biomarkers for several diseases including endometriosis, endometrial and ovarian cancer. The study of the distinctive miRNA expression profiles in these scenarios seems crucial for the clinical diagnosis and/or prognosis of these diseases. In addition, miRNAs could be a useful tool for the treatment of these diseases or they could be implicated in pharmacological treatment responses. Moreover, the development of therapies that modulate miRNA expression, either blocking or mimicking the miRNA activity could represent new therapeutic strategies for any of the aforementioned gynecological disorders.

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**References**


