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REVIEW PAPER

EXTRACELLULAR VESICLES IN THE PATHOGENESIS OF ENDOMETRIOSIS: SCIENTOMETRIC ANALYSIS AND LITERATURE REVIEW

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Summary

Pain and infertility are the most common symptoms of endometriosis (EMS), a gynecological disorder defined as the development of endometrial cells outside the uterus. Extracellular vesicles (EVs) are physiologically active granules that carry molecular content with both diagnostic and therapeutic implications in intercellular communication. We performed a systematic review based on the 2020 PRISMA guidelines to investigate the role of EVs in EMS. On March 3th, 2024, the keywords “endometriosis”, “extracellular vesicles”, “EV”, or “EVs”, along with the terms “treatment”, “monitoring”, or “diagnosis”, were used to search three databases (Medline, PubMed and Scopus). Articles published in English between 2019 and 2024 were counted. 170 EMS patients from 14 studies were included in the analysis. Samples of EVs of various origins were investigated to find possible relevance in the pathophysiology and their diagnostic and therapeutic implications. The most often analyzed cargo were microRNAs. The possible contribution of EVs to pathophysiology has been examined in a number of studies, with an emphasis on their roles in inflammation, angiogenesis, immunomodulation, cell communication, and proliferation. The reports indicated the involvement of EVs in numerous signaling pathways, including the MAP, WNT, TNF and PI3K-AKT pathways.

Keywords: extracellular vesicles, endometriosis, EVs, diagnosis, treatment

Introduction

About 10-15% of women of their reproductive years suffer from endometriosis (EMS), a common gynecological illness marked by the growth of endometrial-like tissue exterior to the cavity of the uterus [1]. The collection of accurate statistical data presents a challenge, as a significant portion of EMS cases remain undiagnosed for extended periods. EMS poses a great burden both for patients and the healthcare system. Women with EMS have significantly impaired quality of life [2]. Furthermore, research showed elevated cancer risk among individuals with the disorder [3]. The diagnosis of EMS can be challenging due to the non-specific nature of its symptoms. The main conditions that require a visit to a gynecologist are fertility issues (EMS may result in the obstruction of the fallopian tubes and formation of antiphospholipid autoantibodies), menstrual cycle disorders, including dysmenorrhea (painful periods), chronic, debilitating pelvic pain that typically intensifies prior to menstruation and dyspareunia (painful intercourse). Moreover, EMS may cause bleeding from the gastrointestinal tract and pain during defecation, as well as hematuria or postcoital bleeding [4,5].

The underlying causes of EMS are not entirely clear. It is believed that EMS is influenced by a combination of both genetic and hormonal factors, as well as environmental influences. One of the most well-supported theories to explain the condition is that of retrograde menstruation, whereby menstrual blood travels back through the fallopian tubes into the abdominal cavity, thereby endometrial cells are able to implant and grow away from the uterus [6-8]. Additionally, there is evidence to suggest that genetic predisposition, immune system dysfunction, and hormonal imbalance play a significant role in the EMS pathogenesis. According to current findings, regulation of the inflammatory response is a pivotal factor in the onset and evolution of EMS, with immune dysfunction being a contributing element in the development of abnormal tissue growth [9-11].

Extracellular vesicles (EVs) have emerged as potential, important contributors in the pathogenesis of EMS and hold promise for both understanding the disease mechanisms and developing diagnostic tools [12]. Extracellular vesicles, also known as exosomes and microvesicles, are small membrane-bound particles that are released by cells and can carry various biological molecules, including proteins, nucleic acids, and lipids [13,14]. The vesicles have been found to be involved in intercellular communication and can transfer their cargo to recipient cells, thereby influencing cellular functions. EVs in EMS have been shown to play a role in the pathogenesis of the disease by facilitating the establishment and growth of endometriotic lesions [15].

Understanding the specific pathways and molecular cargo carried by EVs in EMS is crucial for unraveling the mechanisms underlying the disease and identifying potential therapeutic targets. Moreover, EVs have the potential to serve as a novel minimally invasive diagnostic instrument, thereby reducing the necessity for laparoscopic procedures [16].

Aim of the work

In the review, we aim at consolidating the current knowledge on EVs in EMS, with a focus on their role in pathogenesis and relevant signaling pathways to better understand its mechanisms and identify potential therapeutic targets. Since a reliable marker for the disease is still lacking, EVs may offer a non-invasive diagnostic option, reducing reliance on laparoscopy and improving patients outcomes.

Methods

A systematic review was conducted based on the 2020 PRISMA guidelines [17]. A comprehensive search was conducted on March 3th, 2024 across three major databases: PubMed, Medline and Scopus. The search terms included the following keywords: “endometriosis” and “extracellular vesicles” or “EV” or “EVs” and either “treatment” or “monitoring” or “diagnosis”. The articles were independently reviewed by two researchers (A.W. and M.SK.) at every stage of the evaluation process.

Inclusion and exclusion criteria

The following inclusion and exclusion criteria were applied in order to identify relevant articles for the study. Firstly, articles must have been published in English. Secondly, the publication date range was set from 2019 to 2024. Thirdly, the articles were required to assess extracellular vesicle samples from EMS patients, whether or not they had controls. It was essential that the articles were original papers. Animal studies were excluded from the analysis. Finally, articles exclusively concerning adenomyosis and not EMS were also excluded.

Extraction of data

The authors (A.W. and M.SK.) performed independent evaluations of the reports, in order to compile and extract data. The following information was evaluated for each study: the number of patients and controls, the source of bodily fluids or tissue used to obtain EVs, the techniques employed for isolating EVs, the specific cargo within EVs, the possible diagnostic, therapeutic and pathogenetic implications of EMS, and any identified limitations. It is important

to note that no automation tools were applied during the process, and no supplementary data was obtained from the authors of the selected studies. All extracted data is presented in Table 1.

Table 1. Extracted and summarized data

Study	Number of EMS patients (n=)	Controls (n=)	EV's sample source	EV isolation technique	EV cargo	Pathogenesis	Diagnostic implications	Therapeutic implications	Limitations
Asl et al. [18]	n=5	n=5	Menstrual blood-derived stem cells	Exocib kit (Cib Biotech Co)	Not investigated CD63 and CD81 as exosome-specific markers (flow cytometry)	Altered pathways: VEGF as a major mediator of physiologic and pathologic angiogenesis, cyclin D1 is a cell-cycle regulator, MMP-2 and MMP-9 are markers of migration and invasion, inflammatory factors like IL-6, IL-8, IL-1β, cox-2, NF-kb, HIF1α, and TNF-α, stemness factors like sox2, sall4, oct4, and Nanog, and finally, BCL-2 and Bax are apoptotic genes. Additionally exosomes from the research induced apoptosis in E-MenSCs.	Not discussed	Potential therapeutic effect: proof that mesenchymal stem cells can cure EMS by producing exosomes, E-MenSCs' expression levels of markers linked to inflammation, proliferation, migration, and angiogenesis are decreased by exosomes from patients without EMS (NE-MenSCs).	Limited numbers, in vitro only, EVs cargo not investigated
Hsu et al. [19]	n=3	n=3	Eutopic (Eu) and ectopic (Ec) endometrial	Ultracentrifugation (UC)	Annexin A2 (ANXA2) 36 proteins specific to	EVs-ANXA2 regulates the motility, proliferation, and angiogenic potential of ESCs via the extracellularly regulated kinase	Not discussed	Not discussed, but potential therapeutic implications	Limited numbers, controls are from patients with EMS. The study did not perform purification for non-sEV

			stromal cells (ESCs)		EcESCs-sEVs comparing to EuESCs-sEVs Identified by: Western blotting, NTA and TEM.	(ERK)/STAT3 pathway and pathways related to adherens junctions, cdc42, wnt/β-catenin, actin cytoskeleton, and Rho family GTPases.			protein removal, and did not compare sEV-enriched pellets' activity to sEV-depleted fractions.
Wang et al. [20]	n=6	n=6 endometrial stromal cells (ESCs) without any stimulation	Human umbilical cord mesenchymal stem cells (UC-MSCs)	Ultracentrifugation (UC)	Not investigated, identification of EVs using electron microscopy	EV exposure significantly reduced ESCs' expression of cyclin D1 and MMP-9, while EVs from UC-MSCs inhibited proliferation, invasion, and expression of SF-1, aromatase, and ERb.	Not discussed	UC-MSCs-derived EVs as potential treatment option for EMS	Limited numbers, number of samples not clearly stated
Zhou et al. [21]	n=3	n=3	Eutopic endometrial stromal cells (EuESC) of women with EMS-associated infertility and normal endometrial stromal cells (NESC) of fertile women without EMS	ExoQuick-TC Exosome Isolation Kit (SBI)	A total of 49 differentially expressed miRNA, including 26 up-regulated and 23 down-regulated in EuESC exosomes as compared with NESC exosomes Identified by: transmission electron microscopy (TEM)	HOXA10 and LIF identified as possible targets, (mRNA expression levels significantly decreased in EuESC compared with NESC). In addition, the predicated target genes of these differentially expressed exosomal miRNA were significantly enriched in 76 pathways, including the MAPK and Wnt signalling pathways.	Potential diagnostic implications	Not discussed	RNA seq via RT-qPCR with no validation, limited numbers

Qiu et al. [22]	n=30	n=16	Endometriotic cyst stromal cells (ECSCs) and serum	ECSCs: Total Exosome Isolation Reagent (Thermo Fisher Scientific) Serum: ExoQuick Exosome Precipitation Solution kit	lncRNA aHIF	Exosomal aHIF modulates the proangiogenic behavior of HUVECs (human umbilical vein endothelial cells) and stimulates EMS angiogenesis by activating VEGF-A, VEGF-D and FGF.	Serum exosomal aHIF as a promising biomarker for EMS	Exosomal aHIF as a potential therapeutic target	In vitro only, endometriomas only, ECSCs as the only cell model, proliferative phase samples only, methods imply that the fetal bovine serum (FBS) in the culture media was not depleted of EVs, potentially introducing contaminating EVs.
Huang et al. [23]	Not stated	Not stated	Ectopic endometrial tissues of EMS patients and normal human serum (NHS)	Centrifugation	miR-301a-3p identified by TEM, NTA and western blot	EMS derived exosomal miR-301a-3p mediated macrophage polarization via regulating PTEN-PI3K axis.	Not discussed	Potential therapeutic implications. Downregulation of miR-301a-3p reduces macrophage activity, thus inflammatory response in EMS.	The number of participants not clearly stated as well as the tissue source and methods. Different sources of EVs were used (tissue from lesions from EMS patients and serum from controls). It wasn't demonstrated that EVs contain miR-301-3a.

Khalaj et al. [24]	n=6	Number not specified	EMS lesions, endometrium, peritoneal fluid and plasma, endometrial epithelial carcinoma (EECC), HUVEC and patient-derived endometriotic epithelial (12Z) cell line.	MiRCURY exosome isolation kit (#300,102; Exiqon Inc)	miRNA(miR-206, –29c-3p,–381-3p, –100-5p, –193b-3p, –335-5p, –411-5p –139-3p, –let-7a-3p, –95-3p, –29b-3p, –495-3p, –136-3p, –887-3p,), lncRNA analyzed using next-generation sequencing (NGS) validated using quantitative PCR (qPCR)	EVs from EMS lesions promote angiogenesis, cell growth and pro-inflammatory effects by IL 6, PDGF and macrophage-derived chemokine (MDC). Moreover they carry cargo (miR–30d-5p, miR–27a-3p, and miR-375) that is specific to EMS.	Distinct EV miRNA signatures, miR-30d-5p, miR-27a-3p, and miR-375, are potential diagnostic targets for EMS, distinguishing between patients and controls, and matched lesions.	Not discussed	Limited numbers. Study does not specify number of control sample.
Wu et al. [25]	n=13 (RNA seq n=3, RT-qPCR n=10)	n=13 (RNA seq n=3, RT-qPCR n=10)	CCM of the control endometrium and paired endometrioma and endometrium primary ESCs	ExoQuick-TC Exosome Isolation Kit (SBI)	ATP6V1A, miRNAs, mRNAs (seq), circRNAs, circ_0026129, miR-15a-5p validated with RT-qPCR	Elevated miRNA-15a-5p involved with angiogenesis regulates VEGF-A hence contributing to the pathogenesis of EMS. ATP6V1A is likely involved with cell migration and growth.	Possible diagnostic targets. Network analysis of ceRNAs identified three key components (circ_0026129, miR-15a-5p and ATP6V1A), ATP6V1A correlates with severity and endometrial responsiveness.	Potential therapeutic targets (AJUBA and miR-3187-3p. AJUBA as a negative regulator of the Hippo signaling pathway, which inhibits apoptosis and cell proliferation in EMS).	Small group of patients and controls. Low statistical power with huge number of comparisons and complex displaying calculations.

Feng et al. [26]	n=5	n=6 The samples were divided into two groups (n=6): control and Huc-MScs-exo treatment (10 μg/ml) group	CCM of primary umbilical cord derived MSCs	Exosome extraction kit (#E1310; Bioruo)	No investigation	Huc-MSCs-exo improve endometrial cell migration, increase N-cadherin and Vimentin expression levels, and decrease E-cadherin expression at both mRNA and protein levels.	Not discussed	Huc-MScs-exo as potential treatment option	Limited numbers. The umbilical cord (EV source) and EMS tissue from different patients.
Wu et al. [27]	n=10 (n = 3 for RNA seq, n = 7 for RT-PCR validation)	n=10 (n = 3 for RNA seq, n = 7 for RT-PCR validation)	EMS group: ESCs obtained from ovarian endometriomas and eutopic endometrium control group: ESCs obtained from endometrium	ExoQuick-TC Exosome Isolation Kit (SBI)	miRNA, mRNAs (seq), and lncRNA. Regulatory network expression MIB2 LOC105376166/ miR-214-3p and ADCY3 LOC105371414/ miR-423-5p confirmed using RT-qPCR	Exosomes derived from ESC by transferring competing endogenous RNAs may play autocrine/paracrine roles and promote the pathogenesis of EMs.	Possible use of a panel of EV derived RNA processing for diagnosis as a biomarker.	Not discussed but potential therapeutic implications of lnc-RNA application.	Small number of subjects with very large number of comparisons, selection bias and not the best method to verify the purification of exosomes.

Li et al. [28]	n=26	n=25	Normal/ectopic endometrial tissues and leucorrhea	Differential centrifugation	tRFs and tiRNAs (tRF-Leu-AAG-001) verified using PCR	The tRFs and tiRNAs in ectopic exosomes are enriched in ten pathways, with VEGF and Fc epsilon IR being the most influential. High expression of tRF-Leu-AAG-001 triggers mast cells to express inflammatory factors (IL-6, IL-10, IL-1β, TNF), promoting inflammation and angiogenesis.	Exosomal tRF-Leu-AAG-001 could be a potential EMS biomarker.	Not discussed	Dample size of endometrial tissue not clearly stated, small sample size used for exosomal RNAs extraction and tRFs&tiRNAs sequencing.
Wu et al. [29]	n=42	n=24	Serum	Centrifugation, magnetic separation	miRNA (miR-215-5p and miR-6795-3p, miR-26b-5p) validated with RT-qPCR	Identified MiRNAs may be involved in MAP and PI3K-AKT signaling pathways.	The possible biomarkers miR-26b-5p, miR-215-5p, and miR-6795-3p can be employed to assess the degree of ovarian EMS.	Not discussed	No reports on miRNA chip analysis methods
Huang et al. [30]	n=10	n=10	Plasma	Ultracentrifugation (UC)	50 DE-miRNAs (7 miRNAs upregulated and 43 miRNAs downregulated in the EM patients) miRNA sequenced using microarrays	miRNAs are associated with promoting mesenchymal cell proliferation, TNF and Toll-like receptor signaling pathways, and differentiation of Th1 and Th2 cells.	Blood exosomal miRNAs as potential targets for diagnosing EMS	Not discussed, but potential therapeutic targets	Limited numbers

Zhang et al. [31]	n=9 (5 patients in morphology study, 4 patients in miRNA microarray analysis)	n=10 (5 controls in morphology study, 5 controls in miRNA microarray analysis)	Tubal fluid	Gradient centrifugation and ultracentrifugation (UC)	miRNAs (miR-1273f, miR-5699-5p, miR-6087 and miR-6747-5p) validated by quantitative real-time PCR	Identified miRNAs may be involved in MAPK, Wnt, VEGF and ErbB signaling pathways playing a crucial role in regulating target cell function, affecting cell communication between gametes and embryos and tubal epithelium secretion.	Not discussed	Not discussed	Limited numbers

Methodology for risk of bias assessment

We employed the NHLBI's Study Quality Assessment Tools to evaluate the included studies' risk of bias. Each study was evaluated separately by two independent reviewers (A.W. and M.SK.) to maintain objectivity and reduce potential bias. No automation tools were conducted in the assessment process, ensuring a reliable and comprehensive analysis of the research.

Literature review results

Search results

A total of 14 articles were chosen for inclusion in the final review, following the initial screening process which identified 192 articles. Of these, 129 articles did not align with the specified inclusion criteria (covering the subject of extracellular vesicles, published in English between 2019 and 2024, original papers only). Following the removal of 25 duplicate records, one article was excluded as its focus was exclusively on adenomyosis rather than EMS. Additionally, eight further reports were excluded as they were not relevant to any pathogenesis pathways. The identification process of relevant studies is illustrated in Figure 1.

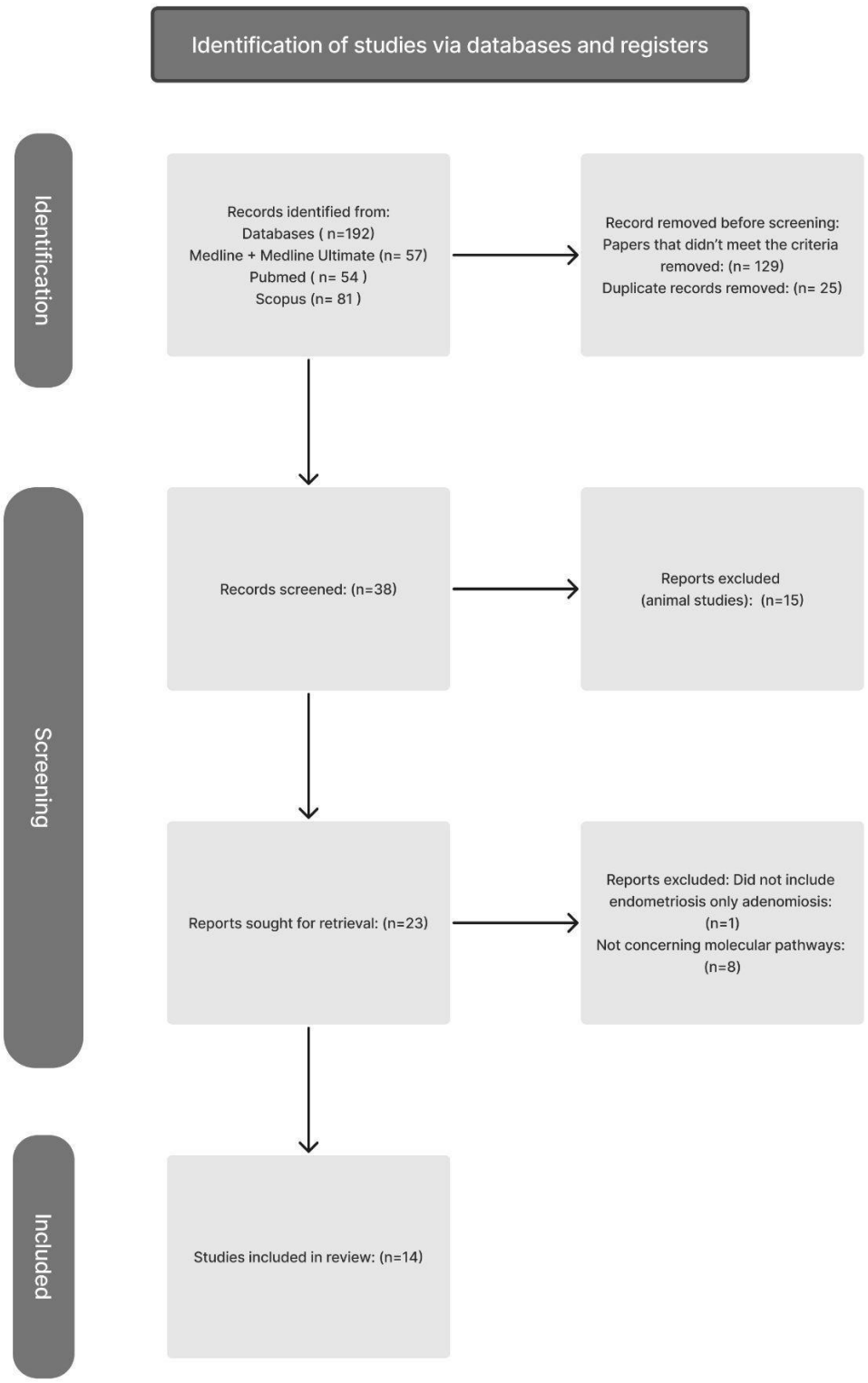

Figure 1. Data identification and screening following the 2020 PRISMA guidelines

Source of extracellular vesicles

The most common EVs' sample sources were eutopic and ectopic endometrial stromal cells (ESCs), which were examined in four of the studies [19,21,25,27] alongside with serum [22,23,29] and EMS lesions [23,24,28], as were analyzed in three of the articles each. Other EVs' sources included menstrual blood-derived stem cells [18], human umbilical cord mesenchymal stem cells (UC-MSCs) [20,26], endometriotic cyst stromal cells (ECSCs) [22], endometrial biopsy [24,28], peritoneal fluid [24], leucorrhea [28], plasma [24,30] and tubal fluid [31].

Isolation and identification of extracellular vesicles

A variety of techniques was employed to isolate EVs. The most common methods of extraction were ultracentrifugation (UC), used in five of the studies [19,20,21,30,31] and commercial kits, which have been employed in a total of seven studies [18,21,22,24-27]. Other techniques included gradient centrifugation [31], differential centrifugation [28] and magnetic separation [29]. The following commercial kits were used: Exocib kit, ExoQuick-TC Exosome Isolation Kit (SBI), MiRCURY, Total Exosome Isolation Reagent (Thermo Fisher Scientific). Identification of EVs was typically conducted through the use of transmission electron microscopy (TEM) and staining with exosome-specific markers, such as CD63 and CD81. Alternatively, Western blotting was employed to confirm the presence of EVs. The cargo within the vesicles was then validated through the use of real-time quantitative PCR (RT-qPCR), qPCR, next-generation sequencing (NGS) or microarrays.

Cargo and function of extracellular vesicles

EVs have been shown to transport special cargo and play a key role in the pathophysiology of the disease by affecting processes such as angiogenesis, cell proliferation, and inflammatory response within the local microenvironment of ectopic, endometriotic lesions. Majority of studies have focused on analyzing the cargo of EVs, which is primarily composed of crucial inflammatory and angiogenic cytokines and micromolecules that are known to be involved in disease progression. Some studies have demonstrated the presence of EVs in specific samples without a thorough examination of their contents [18,20,23,26]. The most frequently identified cargo of the EVs was miRNA, which was reported in a total of six studies [21,25,27,29-31]. In addition, EVs were found to contain the following: lncRNA [22,27,30] mRNAs [25,27,30], circRNAs [25], sRNA [21,24] tRFs and tiRNAs [28], annexin A2 (ANXA2) [19].

EVs role in the EMS pathogenesis

The investigation primarily concentrated on the role of EV-derived cargo in the process of inflammation, angiogenesis, cell migration and cell growth. They participate in the inflammatory response through the differentiation of Th1 and Th2 cells [30], the promotion of macrophage M2 proliferation [23], or the activation of B cells [24]. The reports indicated the potential involvement of miRNAs in numerous regulatory pathways, such as the phosphatidylinositol 3-kinase (PI3K-AKT) signaling pathway [23,29], the mitogen-activated protein (MAP) pathway [21,29,31], the WNT pathway [18,19,21,31], along with the tumor necrosis factor (TNF) pathway [30].

Furthermore, it has been demonstrated that EVs play a role in angiogenesis due to their ability to activate various growth factors, including vascular endothelial growth factor (VEGF-A, VEGF-D) and fibroblast growth factor (FGF) via exosomal aHIF [22], miRNA-15a-5p [25] or tRFs and tiRNAs [28]. Additionally, annexin A2 (sEVs-ANXA2), tRF-Leu-AAG-001 and other miRNAs have been proven to stimulate the growth of blood vessels [19,24,28,29,31].

A number of studies have shown the influence of EVs on cell growth and cell migration. In patients with EMS, exosomes originated from Huc-MSCs were found to increase the levels of Vimentin and N-cadherin at the mRNA and protein levels, decrease E-cadherin expression and dramatically enhance the migration of uterine glandular epithelial cells [26]. Furthermore, ATP6V1A is believed to play a role in cell migration and growth [25] and sEVs-ANXA2 has been shown to regulate the motility and proliferation potential of ESCs via the extracellular signal-regulated kinases (ERK)/STAT3 pathway [19].

Furthermore, it has been demonstrated that numerous pathways are altered in EMS and may potentially be treated with stem cells [18]. The cyclin D1 and MMP-9 expressions in ESCs were significantly decreased by UC-MSCs-derived EVs, as were the SF-1, ERb and aromatase expressions. Therefore, it is possible to achieve the inhibition of ESCs' proliferation and invasion [20]. Ultimately, miRNAs regulate the functions of target cells, influencing communication between gametes and embryos, as well as the secretion and transport activities of the tubal epithelium. The processes consequently affect the health of the female reproductive system [31].

The impact of various EVs and their cargo on pathogenic processes is illustrated in Figure 2.

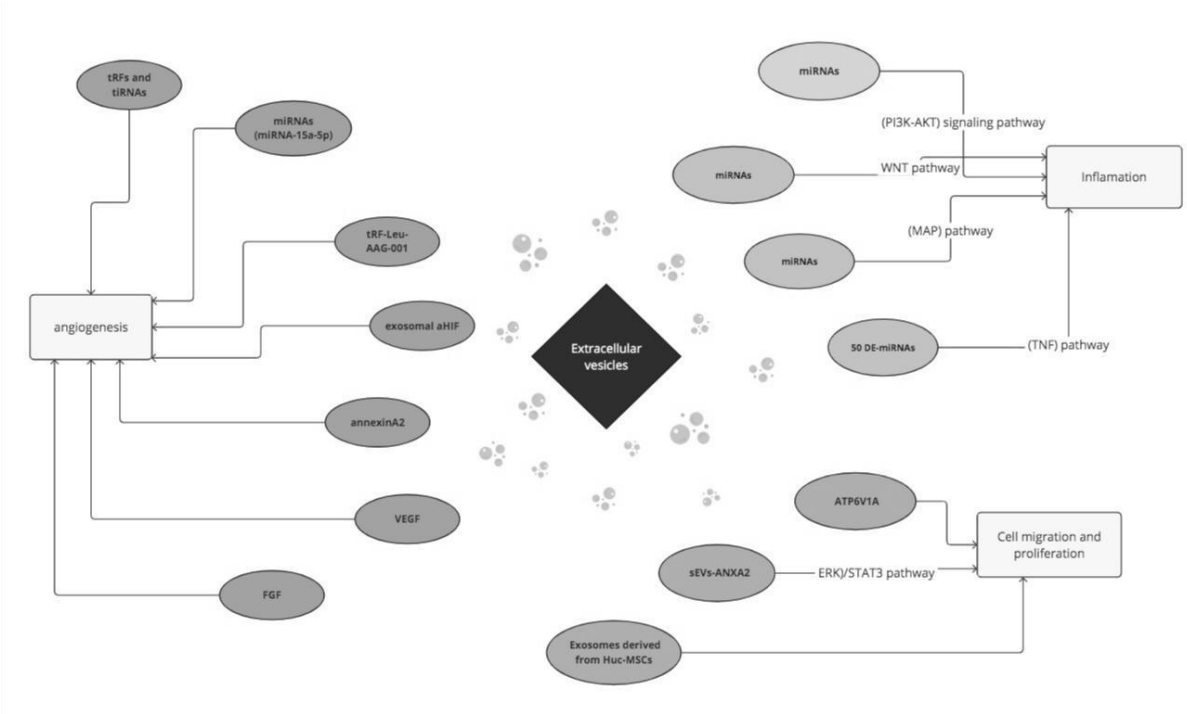

Figure 2. Visualization of EVs' cargo and their role in the pathogenesis of EMS

Therapeutic and diagnostic implications of extracellular vesicles

Two studies have indicated that EVs derived from menstrual blood stem cells and human umbilical cord mesenchymal stem cells (UC-MSCs) may represent a potential treatment option for EMS [18,20]. Exosomes have been demonstrated to inhibit the expression of markers associated with inflammation, angiogenesis, proliferation and migration. One article suggested the potential therapeutic implications of aHIF as a promising target [22]. Another study showed miR-301a-3p overexpression in ectopic endometrial lesions, suggesting that downregulation of the particular mRNA may result in a reduction of macrophage activity and, consequently, an attenuation of the inflammatory response observed in EMS [23]. A number of specific miRNAs [24,27,29,30], serum exosomal aHIF [22], ceRNAs [25] and exosomal tRF-Leu-AAG-001 [28] have been identified as potential biomarkers for the diagnosis of EMS. Furthermore, ATP6V1A

[25] as well as serum miRNA [29] have been demonstrated to correlate with severity and endometrial responsiveness.

Limitations

The specific limitations of each study are listed in (Figure 2). The most frequently observed limitation was the relatively small sample size, with only four of the studies reporting a number of EMS patients greater than ten individuals [22,25,28,29].

Moreover, the number of patients with EMS was not clearly stated in four additional articles [20,23,24,28]. Another common limitation was the absence of appropriate control groups, as well as the use of different sources of EVs between EMS samples and controls [19,20,23,25,26]. A further limitation, observed in three of the studies, was the lack of investigation into EVs' cargo [18,20,26]. As a result, the biological function of the EVs could not be assigned to a specific molecular component. The authors also identified several other limitations, including the potential for selection bias and the necessity for a more effective method for the collection and verification of exosome purification [27].

Discussion

EMS is a common yet disturbing condition for women. Current estimations suggest that between 10% and 15% of women at their childbearing age are affected by EMS, resulting in approximately 190 million individuals impacted [32].

There is disagreement over the pathophysiology of EMS; three basic theories are primarily accepted. The theories include: 1. Menstrual blood reflux to the pelvis; 2. Coelomic epithelium metaplasia; and 3. Endometrial tissue dissemination across the cardiovascular and

lymphatic systems. Moreover, others suggest that genetic factors and modifications in the immune system may potentially contribute to the destructive progression of EMS [33-35].

Although the underlying pathophysiology remains largely unclear, significant progress has been made in understanding progression of the disease.

EMS is characterized by the dysregulation of various signaling pathways (for instance, the vascular endothelial growth factor receptor (VEGF), mitogen-activated protein kinase (MAPK), tumor necrosis factor (TNF), ERK/STAT, WNT, and PTEN-PI3K), which are engaged in biological processes such as angiogenesis, proliferation, migration and apoptosis [19,23,36].

Extracellular vesicles, otherwise termed as EVs, hold molecular cargoes in the forms of proteins, lipids, and nucleic acids, including microRNAs that facilitate processes involved in the development of EMS.

EVs have been demonstrated to participate in the intercellular communication within the endometrial microenvironment. The interaction can alter immune responses, such as Th1 and Th2 lymphocyte differentiation, M2 macrophage amplification, or B cell stimulation, and worsen the inflammation-induced state of EMS. Exosomes derived from mesenchymal stem cells of human umbilical cord (Huc-MSCs) and ATP6V1A are thought to be involved in cell migration and proliferation in EMS patients. Furthermore, it has been demonstrated that EVs promote angiogenesis by activating a number of growth factors, such as FGF and VEGF. Taking into consideration all the facts from the review, we may say that the seemingly distinct pathways engage in intimate cross-talk, where EVs are the connecting binder.

The suggested role of EVs in the pathogenesis of EMS is outlined below. During retrograde menstruation, exosomes released by the endometrial tissue interact with shed cells of endometrium, facilitating their subsequent migration, adhesion, and implantation as well as immune modulation. Furthermore, EVs from freshly implanted ectopic endometrial cells have

been shown to promote further inflammation, angiogenesis and cell proliferation, which in turn leads to the development of EMS lesions. However, it is a hypothesis and yet to be investigated.

Even though it is thought that surgical intervention is the most effective method of dealing with the symptoms related to EMS, the outlook for the use of EVs in medical therapies and diagnostics appears to be very encouraging. EVs have proven to be a potential therapeutic target due to their multiple roles in disease processes. EMS patients treated with exosomes displayed reduced markers of inflammation, which supports the potential for them to be used in treatment for the disease. Furthermore, the presence of exosomes has been detected in various body fluids, including peritoneal fluid, leukorrhea, and plasma; thus they may be useful as easily detectable biomarkers.

Conclusions

In conclusion, there is an ongoing need for clinicians and scientists to collaborate in order to explore new pathways and to conduct studies on EMS therapies. Given the significant role that EVs play in the pathogenesis of the disease, further research is required in order to explore and validate their cargo as potential biomarkers, drug targets, or even treatments to ultimately improve patient outcomes.

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The authors declare that this scientific article was initially prepared with the assistance of the AI tool DeepL Write (https://www.deepl.com/en/write) for grammar and language improvement. The application of this technology was restricted to improving the manuscript's language quality, correctness, and clarity; it did not change the interpretations or substantive content.

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