Endometriosis research in the -omics era

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ABSTRACT

Endometriosis is a pathological condition extensively studied, but its pathogenesis is not completely understood, since its pathophysiology stems from a broad spectrum of environmental influences and genetic factors. Moreover, the nature of this condition is heterogeneous and includes different anatomical entities. Scientists actively pursue discovery of novel biomarkers in the hope of better identifying susceptible individuals in early stages of the disease. High-throughput technologies have substantially revolutionized medical research and, as a first step, the advent of genotyping arrays led to large-scale genome-wide association studies (GWAS) and enabled the assessment of global transcript levels, thus giving rise to integrative genetics. In this framework, comprehensive studies have been conducted at multiple biological levels by using the “-omics” platforms, thus allowing to re-examine endometriosis at a greater degree of molecular resolution. -omics technologies can detect and analyze hundreds of markers in the same experiment and their increasing use in the field of gynecology comes from an urgent need to find new diagnostic and therapeutic tools that improve the diagnosis of endometriosis and the efficacy of assisted reproductive techniques. Proteomics and metabolomics have been introduced recently into the every day methodology of researchers collaborating with gynecologists and, importantly, multi-omics approach is advantageous to gain insight of the total information that underlies endometriosis, compared to studies of any single -omics type. In this review, we expect to present multiple studies based on the high-throughput -omics technologies and to shed light in all considerable advantages that they may confer to a proper management of endometriosis.

1. Introduction

Endometriosis is an enigmatic condition, representing a common, estrogen-dependent gynecological disease. The growth and proliferation of endometrial glands and stroma in ectopic sites characterizes this condition, which appears its most common manifestations in the pelvic cavity (Halas and Arici, 2004; Giudice and Kao, 2004). Various forms of endometriosis have been defined so far, including peritoneal, ovarian, and deep pelvic endometriosis (Zanelotti and Decherney, 2017). Ovarian dysfunction results from mechanisms interfering with folliculogenesis and endometrial receptivity, leading to the final manifestation of the induction of the infertility-based endometriosis (Leyland et al., 2010). Endometriosis affects dramatically the health of women as well as the quality of their life. The gold-standard for diagnosis of this condition refer to laparoscopy and biopsy, while pelvic pain, dysmenorrheal, dyspareunia and impaired fertility represent the main symptoms (Zondervan et al., 2018). Laparoscopy refers to a surgical visual inspection of the pelvic organs, while protocols focused on the treatment of this condition should directed towards a preservation of their fertility (Alborzi et al., 2017).

Endometriosis is a complex disease and various genetic and environmental factors contribute in its development (Simpson et al., 1980; Coxhead and Thomas, 1993; Stefansson et al., 2002). Nevertheless, the exact genetic and pathophysiological basis of endometriosis

Abbreviations: GWAS, genome-wide association studies; NGS, next generation sequencing; WES, whole exome sequencing; WGS, whole genome sequencing; SNPs, single nucleotide polymorphisms; miRNAs, microRNAs; RNAseq, RNA sequencing; SELDI-TOF-MS, Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis

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is still unclear. Genetic studies have revealed many endometriosis-risk loci thus far (Falconer et al., 2007; Nyholt et al., 2012; Rahmioglu et al., 2014). Moreover, a notable impact of epigenetics in endometriosis has been documented last years, thus involving epigenetics in the disease’s development (Wang et al., 2008; Tan et al., 2011).

Advances in the modern technologies and bioinformatics have resulted in the generation of large-scale biological data sets, the so-called -omics data and, as a consequence, biomedical sciences were proceeded the –omics century. Remarkably, since the turn of the century, a definite increase of techniques using omics technologies has been reported. “omics” approaches in endometriosis patients, including transcriptomic and proteomic analysis of blood (Vicente-Munoz et al., 2015) and endometrial fluid (Ametzazurra et al., 2009) have been already used to evaluate the disease severity and, in fact, endometrial fluid has been proven to be an excellent body fluid allowing the development of new, minimally-invasive diagnostics for any endometrial disease or even to predict implantation (Vilella et al., 2013). Moreover, other areas of modern sciences, including epigenomics and metabolomics are moving towards the omics scale, thus being promiscuous of an interdisciplinary data integration that may lead to a better understanding and management of endometriosis.

This review cuts across the boundaries between genomics, epigenomics, transcriptomics, proteomics, metabolomics, lipidomics, secretomics and microbiomics, aiming to summarize endometriosis -omics data that have been generated and analysed (Fig. 1) and shed light on the potential strengths and weaknesses of this global approach.

2. Genomics of endometriosis

Genomics refers to the study of genomes, that is the complete genetic makeup of an organism, as well as the total number of genes contained in these genomes. It is considered as the most mature field of the –omics technologies and, especially in the medical research, the main focus of genomic studies deals with the identification of genetics variants that can be associated with the development of a complex disease, the response to a pharmaceutical therapy and the prognosis of the future situation of a patient. Until now, hundreds of valuable genetic markers have been developed, in multiple racial and/or ethnic populations, associated with various multifactorial diseases. Importantly, Genome Wide Association Studies (GWAS) in combination with high-throughput technologies, including genotype arrays, next generation sequencing (NGS) for whole genome (WGS) or whole exome sequencing (WES) have contributed greatly to the revolution of medical research (Hirschhorn and Daly, 2005; Koboldt et al., 2013).

Despite the significant progress into the pathophysiology of endometriosis and the various theories suggested so far, the precise aetiology is still unknown. As a complex disease, endometriosis is caused by interactions of various genetic and environmental factors, with each of them exerting a modest effects on risk for this situation. Based on twin studies conducted, the heritability of endometriosis has been estimated at approximately 50% (Treloar et al., 1999; Saha et al., 2015). The high prevalence of endometriosis among related versus unrelated women strongly suggested a familial association of endometriosis, which has been documented in various studies (Coxhead and Thomas, 1993; Nouri et al., 2010). Furthermore, a strong familial aggregation has also been found by studying a large pedigree of non-human primates (Zondervan et al., 2004). Linkage analysis studies, aiming to the investigation of allelic co-segregation at a suspect disease-associated genetic locus, have been widely used for the identification of genomic regions harboring various polymorphisms related to risk for endometriosis (Krishnamoorthy and Decherney, 2017). Thus, linkage analysis studies have detected regions on chromosomes 10q26, 20p13 and 7p15.2 as harbouring polymorphisms leading to familial endometriosis (Treloar et al., 2005; Zondervan et al., 2007).

Gene association studies take into account candidate genes and single nucleotide polymorphisms (SNPs), aiming to unravel the relation of genetic variation with the development of the disease. Hundreds of candidate gene association studies have been performed thus far, by examining putative genes of interest, with the majority of them not producing replicable results (Rahmioglu et al., 2015). Thus, various polymorphisms located in hormonal function-, angiogenesis-, inflammation-, autoimmunity-, cellular cycle-, proliferation- and apoptosis-related loci as well as detoxification and tumor growth/suppression genes have been associated with endometriosis (for a review see Vassilopoulos et al., 2019). Many disease-associated loci have been identified through GWAS and meta-analyses performed in samples of different ethnic and/or racial origin, with these loci being implicated in the regulation of cell cycle and transcription, in inflammation, cell adhesion and signaling as well as in oxidative stress and metabolism (Kobayashi et al., 2014; Sapkota et al., 2017; Ulmari et al., 2017; Rahmioglu et al., 2017). Notably, the number of novel endometriosis-associated loci is increased because new GWAS and meta-analyses are presently in progress. Eight genome-wide association studies (GWAS) in women of European and East Asian ancestry have been published to date (Zondervan et al., 2018) and they have identified 19 distinct disease-associated signals harbored at 14 loci (Sapkota et al., 2017). Interestingly, most of the conducted GWAS have highlighted that the signals for most loci are more clear and distinct at stage III/IV disease (Revised American Society for Reproductive Medicine classification of endometriosis, 1996. 1997; Sapkota et al., 2017), thus suggesting that these loci affect mainly the advanced stage of endometriosis, which are associated with a higher disease’s severity. Genes located nearest to the risk loci or harbouring them are referred to GREB1, VZFT, FNI, IL1A, LINC00339-WNT4, KDR, SYNE1, CDKN2B-AS1, PARP1P2, CCDC170, CDC42 and FSHB (Doherty et al., 2010; Nyholt et al., 2012; Rahmioglu et al., 2014; Sapkota et al., 2017; Hodgkinson et al., 2018). It is worth noting that most loci detected to be associated with endometriosis, as is typical for GWAS results, reside in inter-genic regions or other non-coding genomic regions and, as a consequence, functional experiments combined with integration of genetic, transcriptomic and epigenetic data is required in any attempt to clarify the functional significance of the polymorphisms identified.

3. Epigenomics in endometriosis

Epigenomics deals with the study of heritable changes in gene function that are not associated with changes in DNA sequence as well as the reversible modifications of DNA-associated proteins, such as DNA methylation or histone acetylation. Epigenomic mechanisms affect all genomic regions in DNA that are packaged together with proteins (e.g., histones) to give raise to a structure known as chromatin. DNA methylation (in cytosine of GC dinucleotides) and histone modification are the most characterized epigenomic modifications. Epigenetic modifications regulate gene expression without altering the DNA sequence. The importance of epigenetic modifications in various biological

![Fig. 1. A schematic diagram of the cumulative number of -omics publications in endometriosis used for the preparation of the present article.](image)
processes, including developmental, physiological and pathological ones, and diseases' developmental mechanisms has been pinpointed thus far as it raised by aberrant DNA methylation studies as well as many epigenome-wide association studies that have been conducted (Kim et al., 2010; Horvath 2013). There is accumulative evidence that methylation alters certain genes, thus contributing to the pathogenesis of endometriosis, while the broader impact of epigenetics in endometriosis has been extensively approached in the recent years (Zelenko et al., 2012; Izawa et al., 2013). Thus, DNA-methylation data may have the potential to uncover molecular mechanisms leading to endometriosis.

Various epigenetic studies have focused on differential DNA methylation in single genes or genome-wide range but histone modifications have not been well investigated in endometriosis (Dyson et al., 2014). Thus, genome-wide differences in DNA methylation between endometriotic and normal endometrial stromal cells have been reported and in a study performed by Dyson et al. (2014) a total of 403 genes revealed significant differences as regards with the methylation pattern, with a high percentage of these genes encoding transcription factors and implicated in the pathogenesis of endometriosis, i.e. the HOX gene clusters, nuclear receptor genes, and the GATA family of transcription factors. Particularly, a higher methylation rate was shown in endometriotic stromal cells in comparison with the endometrial stromal cells throughout the promoter and coding region of GATA-binding factor-6 (GATA6), whereas exons 2 and 4 of GATA6 showed full methylation in endometrium and less methylation in endometriosis. This data indicated a differential regulation of the expression of hundreds of genes in endometriotic and endometrial stromal cells through DNA methylation. Steroidogenic factor 1 (SF1) is an orphan nuclear receptor that is totally absent in endometrial stromal cells but shows a 12,000-fold higher presence in endometriotic stromal cells. SF1 was found to be heavily methylated in endometrial stromal cells (Xue et al., 2007a). Moreover, the progesterone receptor B (PR, encoded by the PGR gene) was found to be differentially methylated in endometriotic vs endometriotic stromal cells, thus resulting in the suppression of its expression in endometriotic cells (Dyson et al., 2014). ERβ encodes estrogen receptor 2, which is considered as a key mediator of estrogen action in endometriotic stromal cells. It is deregulated due to altered methylation in ectopic endometrial tissue compared with eutopic tissue (Bulun et al., 2015). Thus, a remarkably higher ERβ mRNA and protein expression was observed in endometriotic stromal cells compared to the normal endometrium, due to hypomethylated promoter region in ESR2 gene (Xue et al., 2007b; Monsivais et al., 2014). ERβ (ESR2) levels were found to be 142-fold higher compared with normal endometrium (Xue et al., 2007b). Given that the ectopic endometrium of women with endometriosis appears higher ERβ expression compared with the endometrium of healthy women, it has been suggested that aberrantly high levels of ERβ in the endometrium may play a role as a susceptibility factor for the development of endometriosis (Bulun et al., 2019).

In several other genes, which have been associated with endometriosis, remarkable variations in DNA methylation have been found so far, including E-cadherin (Li et al., 2017), cyclooxygenase-2 (Zidan et al., 2015) and Homeobox A10 (Wu et al., 2005) genes. In a recent study, performed in a Northern Chinese population, was shown that aberrant methylation of the IL-12B promoter region may be responsible for the significantly increased mRNA levels observed in ectopic and eutopic endometrium of patients with ovarian endometriosis, thus being implicated in the development of this condition (Zhao et al., 2019).

Studies applying microarray-based DNA methylation analysis to eutopic endometria of endometriosis patients have been performed and many aberrantly methylated genes were identified, including MGMT, DUSP22, CDCA2, ID2, TGF531B, ZNF681, IGSF2I, MAFB, HOXD10 and HOXD11 (Naqui et al., 2014). Moreover, Saeed et al. (2016) did not find distinct differences in the endometrial DNA methylation profiles between endometria of patients and controls. Notably, DNA methylation profiles were highly influenced by the menstrual cycle phases. In another study conducted by Houdbaran et al. (2016), focusing on the comparison of endometrial DNA methylation patterns and associated gene expression levels in patients with endometriosis versus controls, in different menstrual cycle phases, a small number of differentially methylated loci between the two groups was found.

4. Transcriptomics in endometriosis

Transcriptomics, also know as functional genomics, deals with the analyses of gene expression patterns, the so-called transcriptome. The transcriptome consists from a set of mRNA that is produced within a cell at a particular time and under specific conditions. Transcriptomics examines the RNA levels in the whole genome both qualitatively and quantitatively. Transcriptomic studies have shown last years that although about 3% of the genome encodes proteins, up to 80% of it is transcribed (Consortium EP 2012).

Last years, microarrays-based genome wide analysis enabled the simultaneous analysis of the expression levels of thousands of genes. As concerns with the endometriosis studies, this methodology was used for the identification of differentially expressed gene in ectopic endometrium versus the corresponding eutopic counterpart or the healthy endometrium. Numerous studies have detected genes with altered expression in ectopic endometrial tissue compared to eutopic endometria, including genes that belong to RAS, MAPK, and PI3K signalling pathways (Giudice et al., 2008) or other such as CHEK1, ERBB family, laminin gamma, and Ki-67, which are associated with immunological, neurocrine, and endocrine functions (Khan et al., 2012). Furthermore, in other studies, Monsivais et al. (2012) and Suryawanshi et al. (2013, 2014) found various dysregulated genes belonging mainly to pathways involved in the regulation of metabolism and the action of protaglandins and glucocorticoids or complement pathway, respectively. In a recent study performed by Ahn et al. (2016), many differentially expressed genes were detected, involved in cellular adhesion, immune cell recruitment, T-cell cytotoxicity, apoptosis and cell signalling.

In transcriptome studies aiming to answer the question whether there are any differences between endometria of patients with endometriosis and healthy women, numerous genes were identified whose expression levels were up- or downregulated, while the gene expression differences were found to be related with the endometriosis' stage (Kao et al., 2003; Matsuzaaki et al., 2005; Fassbender et al., 2012a,b). Thus, analysis of differently expressed genes in endometria of patients with severe endometriosis exhibited dysregulation of various pathways, which have been already associated with endometriosis in previous studies (Giudice et al., 2008). Similarly, dysregulation of the RAS/RAF/MAPK and PI3 kinase signalling pathway genes was observed in other studies (Aghajanova and Giudice, 2011; Fassbender et al., 2012a; Zhou et al., 2016), thus linking the detected pathways with pathogenesis of the disease.

Eyster et al. (2007) attempted to identify differentially expressed genes in eutopic endometrium compared with ectopic endometrium by using DNA microarrays and analyzing 11 patients with endometriosis. Genes that were expressed differentially included genes coding for proteins associated with the immune system and inflammatory pathways, cell proliferation, cell adhesion and components of signal transduction pathways. Aghajanova and Giudice (2011) analyzed the transcriptome of eutopic endometrium across the menstrual cycle of severe (stage III/IV) versus mild (stage I/II) endometriosis, by analyzing 19 women with mild and 44 with severe endometriosis. Comparison of differentially regulated genes revealed dysregulation of progestone and/or cyclic adenosine monophosphate (cAMP)-regulated genes and genes related to thyroid hormone action and metabolism. The extra-cellular matrix proteoglycan versican (VCAN) was the most highly expressed gene in severe disease. Interestingly, an upregulation of microRNA 21 (miR21) and Dicer1 transcripts was also detected, thus suggesting a potential role for microRNAs (miRNAs) in the pathogenesis.
of severe versus mild endometriosis. Moreover, Crispi et al. (2013), aiming to identify molecular changes involved in endometriosis, proceeded in a comparison of the transcription profile of the ectopic and eutopic endometrium of the same patients with respect to endometrium from unaffected women. About 120 genes were found with a clear opposite behaviour between ectopic and eutopic samples. Various genes were identified to be specifically deregulated in the ectopic endometrium, including LEFTY2, and GREM1 genes that are related to embryological developmental failure. Other genes detected, such as THBS2 and THBS1, play a role in cell–cell interaction (Agah et al., 2002), while SERPINE1 and SERPINE2 are involved in tissue remodeling (Lee et al., 2011). In analyses conducted in peripheral blood by De Sanctis et al. (2011) and Mabrouk et al. (2012) aiming to assess the mRNA levels of genes that were upregulated in ectopic endometrium, MMP-3 levels of mRNA were increased while the ones of survivin were decreased in endometriosis patients versus controls (De Sanctis et al. 2011).

Importantly, new markers are in the horizon. MicroRNA have have been considered as potential noninvasive biomarkers for endometriosis. Numerous microarray-based miRNA studies concentrating on eutopic endometrium have been performed thus far. MicroRNAs (miRNAs) are short, single-stranded noncoding RNAs, that contain on average 22 nucleotides, constituting an important category of gene-expression modifying molecules. The pairing of miRNAs with homologous sequences leads to the inhibition of their transcription or translation. According to the function of miRNAs, downregulated levels of a miRNA lead to an upregulation of its target mRNA translation. MicroRNAs are involved in several pathophysiological processes, including angiogenesis, apoptosis, proliferation, differentiation and matrix remodeling (Braza-Boïls et al., 2014). Importantly, several studies have shown a potential role of numerous miRNAs in the pathogenesis of endometriosis, through their involvement in major physiological processes such as angiogenesis, apoptosis and estrogens synthesis (for a review see Borghese et al., 2017). In a study conducted by Wang et al. (2013), the levels of miR-199a and miR-122 were found to be upregulated while miR-145*, miR-141*, miR-542-3p and miR-9* appeared downregulated when samples from patients and controls were compared. In another study focused on eutopic endometrial from cases and controls, aiming to detect a potential disease-specific endometrial miRNA signature, Burney et al. (2009) found six miRNAs expressed in lower levels in eutopic endometria of patients. Shi et al. (2014) reported 36 downregulated miRNAs in endometrial of patients. Moreover, Laudanski et al (2013) found that miR-483-5p and miR-629-3p, involved in the regulation of IGFB and inflammation, respectively, were downregulated in the eutopic endometrium of women with endometriosis in comparison to controls. Furthermore, Laudanski et al (2015) found three miRNAs to be upregulated in the eutopic endometrium of patients with endometriosis compared with the healthy women.

The miRNA expression was found varying across the menstrual cycle (Ohlsson Tengue et al., 2009) and, particularly, miRNAs targeting several cell cycle regulators were found to be over-expressed in the secretory phase (Kuokkanen et al., 2010). In this framework, Mari-Alexandre et al (2018) found higher levels of miR-106b-3p, -451a, -486-5p in women with endometriosis compared to controls when defined the miRNA levels and its relationship with cytokines content in peritoneal fluid (PF). More specifically, these women presented in menstrual phase up-regulation of miR-106b-3p, -130a-3p, -150-5p, -185-5p, -451a and -486-5p in comparison with other phases, while control women did not.

Considering the inconsistency observed between the results of more that 200 relevant studies that have been performed so far (Saare et al., 2017), the application of the reported miRNAs as markers for endometriosis is still obscure and obviously limited. Therefore, a deep knowledge of the endometrial miRNAome has to be gained in order the pathophysiological significance of the candidate miRNAs to be unraveled.

With the advent of transcriptomics and Next Generation Sequencing (NGS), RNA sequencing (RNAseq) has been applied in studies of endometriosis. RNAseq has the ability of the sequencing and quantification of millions of RNA fragments and, in combination with bioinformatics, represents an invaluable tool for a complete transcript read. Thus, Zhao et al. (2019) aimed to identify candidate pathogenic genes and biomarkers of endometriosis using mRNA sequencing. Differentially expressed genes (DEGs) were identified and by using RNAseq it was found that matrix metalloproteinase 11 (MMP-11), dual specificity phosphatase 1 (DUSP1), Fos proto-oncogene and serpin family E member 1 (SERPINE1) were found overexpressed while adenosine deaminase 2 (ADA2) was found significantly lower in the eutopic endometrium of women with endometriosis. Moreover, Predeus et al. (2018) attempted to detect basic differentially expressed genes in endometrial lesions in comparison to eutopic endometrium of the patients, during mid-secretary phase, by using RNAseq and subsequent RT-qPCR analysis. Several genes were upregulated in eutopic endometrial lesions, including PLA2G2A, alcohol dehydrogenase 1B (ADH1B) and fatty binding protein 4 (FABP4). Of note, the last two genes are key genes in basic metabolism and known for their function in adipose tissue.

5. Proteomics in endometriosis

Proteomics deals with the studies of the changes in all proteins expressed and translated from a single genome (James, 1997) and refers to all proteins released into the surrounding biological fluid (Hein et al., 2013). The high number of proteins expressed by a cell or an organism vary from cell to cell and are dependent on the interaction of many factors. From this viewpoint, proteomics is believed to be substantially more complex than genomics, considering that the genome of an organism remains mostly unchanged.

Proteomics research is based on the detection of differentially expressed protein/peptides in different tissues and/or conditions. These changes in protein expression may be either a precursor to endometriosis or consequence of this disease (Siristatidis, 2009). Thus far, differentially expressed protein or peptides have been identified between patients with endometriosis and controls in blood and urine as well as comparing eutopic with ectopic endometrium (May et al., 2011). Moreover, several proteins have been revealed when compared the eutopic endometrium from healthy women with patients with endometriosis but a few of them have been validated for a potential role in the etiology of endometriosis (Siva et al., 2014).

In a comparative study, focusing on the identification of proteins expressed in eutopic endometrium and sera of women with endometriosis in different stages of the disease vs. controls, Zhang et al. (2006) found that eleven proteins were differentially expressed. Furthermore, when Ten Have et al. (2007) studied the differential protein expression profile of the eutopic endometrium of women with and without endometriosis in the secretory phase of the cycle, they found a total of 119 proteins to be differentially regulated between endometriotic and control tissue. These proteins were enrolled in various cell pathways and functions, including apoptosis, immunity, cell structure and transcriptional regulation.

A non-invasive test for endometriosis has not been presented and confirmed upon now. However, in line with the growing evidence regarding the necessity of an early non-invasive test for the diagnosis of endometriosis, extended investigations of the proteomic content of the endometrial fluid (EF) from women with endometriosis have been conducted. Thus, Ametazzura et al. (2009) examined endometrial aspirate, exhibiting a complex proteome composition with more than 800 protein spots. Among these differentially expressed proteins, cytoskeletal proteins are highly represented. Moreover, changes were observed between women with endometriosis and controls in proteins involved in signal transduction and cell cycle regulation, as well as a number of
different enzymes involved in various metabolic pathways. All this data are beneficial in order to identify proteins implicated in endometriosis.

Zheng et al. (2011) analyzed serum samples and identified three peptide peaks (5988.7; 7185.3, 8929.8 m/z) that distinguished women with endometriosis vs. healthy ones. Fassbender et al. (2012b) analyzed plasma samples instead of serum ones and reported 18 peptides/proteins in different levels in women with endometriosis versus control ones. Furthermore, El-Kasti et al. (2011) used the same experimental platform to investigate urine samples and identified six peptides that were able to discriminate patients with endometriosis at stages III or IV vs. patients without the condition. In addition, using MALDI-TOF and LC-MS/MS technology, Wang et al. (2014) identified five peptides exhibiting significantly higher levels in patients with endometriosis compared to controls. Tokushige et al. (2011) managed to show twofold higher levels for 10 proteins and twofold lower levels for 60 proteins. However, these results need further validation because of the small number of subjects enrolled in this study. Twenty-two urine proteins at higher levels in endometriosis patients were detected by Cho et al. (2012), with vitamin D-binding protein exhibiting the highest differential expression. In an exploratory study, Kyama et al. (2011) and Fassbender et al. (2013) identified two upregulated mass peaks, corresponding to T-pestin and annexin V proteins, thus allowing the identification of endometriosis with maximal sensitivity and specificity.

In a recent proteomic study of eutopic and ectopic endometrial tissue samples from cases controls, performed by Irungu et al. (2019), proteomic changes associated with cycle phase and endometriosis were identified in eutopic tissue from over 1400 identified proteins. Muscle-related proteins were highly expressed in ectopic versus eutopic tissue, while extracellular matrix (ECM) proteins were also highly expressed in ectopic tissue. Moreover, it was found that the best single marker for discriminating endometriosis from healthy women remained CA-125 (cancer antigen 125), which is a glycoprotein detected in human uterine fluid.

6. Metabolomics in endometriosis

Metabolomics is a research field focusing on the development of methods to analyze low molecular weight compounds, including amino acids, fatty acids, carbohydrates, or other products of cellular metabolic functions in various biological systems. It was initially defined as the quantitative measurement of perturbations in the metabolite complement of individual cells or cell types in response to some stimuli or due to disease, drug administration, or different growth conditions (Nicholson and Wilson, 2003). Metabolomics includes the detection and quantification of low molecular weight molecules i.e. in a human subject. And is defined as a group of small metabolites found in a biological sample, that indicates the cellular activity (Fauser et al., 2011). The metabolome represents one of the metabolites compared to controls. Recently, Domínguez et al. (2017) conducted a lipidomics analysis by using endometrial fluid, in a case-control study for the lipid profiling and they found that sphingolipids monohexosylceramides and ceramides exist in lower levels in the endometrial fluid samples of women with endometriosis, thus suggesting the likely crucial role of these lipids in the disease. Furthermore, glycerolipids and glycerophospholipids were over-represented in the patients, while increased levels of acyl carnitines were observed in the endometrial fluid of women suffering from ovarian endometriosis.

7. Lipidomics in endometriosis

Lipids are hydrophobic or amphipathic molecules characterized by unique structural and biological properties and they are important as signaling molecules that are involved in various cellular processes (Kim et al., 2010). Metabolomics and lipidomics are integral parts of systems biology and represent two interdisciplinary fields focusing on the study of complex interactions in biological systems. They are useful tools in systems biology in any attempt to understand the metabolites and lipids that are present in various samples of biological origins and give information to scientists regarding the underlying mechanism of a given disease (Fiehn, 2001). Lipidomics is a subset of metabolomics, referred to the time-related or stimuli-dependent changes in the quantity of lipids. Thus, observed quantitative differences, due to dysregulation of lipid metabolism, can be used to better characterize phenotypes and biological responses to diseases as well as genetic modifications and pharmacological treatments (Fiehn, 2002).

Previous studies of Lee et al. (2016) had detected alterations in the sphingolipid metabolism flux in endometrial tissue of women with endometriosis. Yvouk et al. (2012) studied the serum of patients with ovarian endometriosis and described eight differential lipid metabolites compared to controls. Recently, Domínguez et al. (2017) conducted a lipidomics analysis by using endometrial fluid, in a case-control study for the lipid profiling and they found that sphingolipids monohexosylceramides and ceramides exist in lower levels in the endometrial fluid samples of women with endometriosis, thus suggesting the likely crucial role of these lipids in the disease. Furthermore, glycerolipids and glycerophospholipids were over-represented in the patients, while increased levels of acyl carnitines were observed in the endometrial fluid of women suffering from ovarian endometriosis.

In a lipidomic analysis conducted by Cordeiro et al. (2015), who enrolled women with endometriosis and controls, sphingolipids and phosphatidylcholines of the follicular uid (surrounding the developing oocyte) (Cordeiro et al., 2015), urine (Vicente-Munoz et al., 2015), serum (Dutta et al., 2012) and plasma (Lee et al., 2016). All these previous studies had shown substantial changes in their metabolite profile. Thus, many metabolites from different chemical classes of altered tissue metabolites were detected and further investigation showed that some of them appeared significant altered concentrations in various stages of endometriosis (Dutta et al., 2018).

8. Secretomics in endometriosis

Secretomics refers to the global study of proteins that are secreted by a cell, tissue or organism at any given time or under certain conditions (Hathout, 2007). The proteins constituting the secretome regulate various biological and physiological processes including cell signaling, matrix remodeling and metastasis of malignant cells (Pavlovi and
Diamandis, 2010). Therefore, they are of special importance to gain insight of clinically relevant biomarkers and putative therapeutic targets.

It has been suggested that secretomics may be proven beneficial for the assessment of endometrial maturation and receptivity (Berlanga et al., 2011). In this respect, secretomics may be used for the screening of the molecular content in human endometrial fluid, considering that the important role of endometrial secretions in acquisition of endometrial receptivity and embryo implantation is well documented so far (Salamonsen et al., 2009). Moreover, data has presented referred to correlations between the uterine secretory activity and the reproductive outcome (Bhusane et al., 2016). Thus, deficiencies of uterine secretome have been associated with infertility (Dimitriadis et al., 2006), while investigation of human uterine fluid has been suggested that may gain insights into recurrent pregnancy losses, and other endometrial pathologies that also appear as clinical manifestations of endometriosis. Although there is not clear endometriosis-associated data regarding secretomics so far, further investigation of cell or tissue secretomes involved in the development of the condition holds great promise for the identification of new endometriosis markers and therapeuic targets.

9. Interactomics in endometriosis

Interactomics refers to the global relationships among genes, proteins, ligands and metabolites. It is a discipline at the intersection of bioinformatics and biology, which deals with the study of the interactions as well as of the consequences of these interactions between proteins and/or other molecules of a cell (Kiemer and Cesareni, 2007). Importantly, it has been suggested that cellular networks underlying genotype-phenotype relationships may be predictive of novel, yet unidentified genes involved in human diseases (i.e. ataxia-associated genes) (Vidal et al., 2011). However, no interactomics-associated data related to endometriosis are available upon now.

10. Microbiomes in endometriosis

Microbiomics investigates together all the microorganisms of any given community. Microbiota consist of all microorganisms that colonize human mucosal surfaces, skin and gut, including bacteria, fungi and viruses, while their genes constitute the microbiome (Org et al., 2015). Notably, dysbioses in the microbiome have been associated with numerous diseases (Petersen and Round, 2014; Trompette et al., 2014). The human microbiome is enormously complex and the identification of microbial constituents causing diseases is very complicated due to the high degree of diversity among persons (The Human Microbiome Project Consortium, 2012).

How the microbiome influences woman health and endometriosis, and how human and microbial genetics interact are areas of considerable interest. It has been suggested that the microbiome may influence endometriosis, given that recognizable microbial families have been found in the reproductive tracts of women that are at reproductive-age (Chen et al., 2017). Distinct differences have been identified in the cervical and uterine microbiome between women with endometriosis and healthy ones (Cregger et al., 2017).

Thus, Chadchan et al. (2019) presented results suggesting that gut microbiota promote endometriosis disease progression and antibiotic therapies was found to reduce endometriosis progression in mice. Moreover, another indication for a possible link between the gut microbiota and endometriosis is the observation that women with a high omega-3 polyunsaturated fatty acids (PUFAs) intake exhibit a lower risk for endometriosis (Missmer et al., 2010), a finding that may be explained partly by changes in the gut microbiome that are induced by diet (Laschke and Menger, 2016). Furthermore, the gut microbiota may leads to endometriosis through its role in the regulation of estrogen cycling (Flores et al., 2012). Interestingly, in another study was found an association between the gut microbiota and the chronic stress level in endometriosis patients (Xu et al., 2017). Particularly, the levels of Paraprevotella, Odoribacter, Veillonella and Ruminococcus were found significantly reduced in chronic stressed endometriosis patients.

The human body comprises a diverse microflora that is established early in life (Clemente et al., 2012). The microbial flora includes the female reproductive tract, notably the vagina, where Lactobacilli species are the most dominant species, producing lactic acid that controls the low pH of the vagina (Ravel et al., 2011). Of note, the composition of the vaginal microbiome was found to be dependent on hormonal changes that are associated with the menstrual cycle (Gajer et al., 2012). In a study by Verstraalen et al. (2016), who examined the microbiota of the endometrium, 183 different bacterial phylotypes were identified. Moreover, in an attempt to delineate the role of microbial dysbiosis in the development of endometriosis, it was pointed out that the imbalance of key microbes identified in women with endometriosis, may lead to inflammatory property of endometriosis (Puca and Hoyne 2017).

When analyzed bacterial communities from various sample sites, significant differences were found between the uterus, cervix and stage III endometriosis, thus suggesting a plausible useful diagnostic application of bacterial community profiling for identification of endometriosis (Cregger et al., 2017). The identification of a specific bacterial community profile, associated with endometriosis, may serve as a valuable, novel, early diagnostic tool, which will determine the bacteria that are associated with the disease and help further to restore healthy bacterial populations. As a consequence, this may reduce inflammation and pelvic pain that accompany endometriosis and, probably, restore fertility.

11. Conclusions and future perspectives

Since the turn of the century there has been an explosion of research using -omics technologies (Simons, 2018) and it is undoubtedly expected that they will be widely employed soon for the investigation and diagnosis of endometriosis. All -omics should contribute to the identification of new therapeutic targets, prediction of response to therapy, personalized treatment protocols and better therapeutic outcome of the disease. Despite the promising direction there are a number of pitfalls that should be avoided in this type of studies. Most -omics technologies suffer from the serious problem of reproducibility (Pulverer, 2015) due the fact that scientists have failed to develop methodologies that are quantitative, reproducible, and, most important, comparable. So, if the -omics data that are produced are not quantitative and reproducible, they obviously lack comparability and their contribution to systems biology tends to be meaningless, unreliable and unable to gain insights how biological machineries work and diseases are developed (Simons, 2018).

Although GWAS play a primary role concerning the unraveling of the genetic contribution in the development of endometriosis, the reliability of their findings in some cases is lowered due to a certain bias regarding their design, the phenotypic characterization of the individuals recruited and the reproducibility of the results. Thus far, genetic risk factors for endometriosis and translation of genetic findings have been successfully used for getting improved clinical outcomes. Transcriptome analysis that complements genomics by allowing to decipher gene expression, has contributed to the discovery of molecular networks responsible for disease activity and disease subtypes, while it keeps the promise to be beneficial as an approach for disease; s prognosis. The evolving proteomics technologies, focusing on the comprehensive analysis of an individual’s proteins, give the promise that protein profiles that are associated with endometriosis will offer a unique opportunity towards the establishment and development of proteomics-based assays for early diagnosis and treatment (Siristatidis, 2009). Furthermore, metabolomics may possibly help in the exploration and better understanding of underlying biochemical pathways
associated with endometriosis (Bengtsson et al., 2016). Comparison of the lipid profile from women with endometriosis and healthy can be proven a powerful diagnostic tool, which may facilitate further development of disease’s monitoring techniques, precise diagnosis, new treatment strategies and, potentially, an individualized medicine treatment. A better understanding of the healthy microbiome will help enormously to the direction of the delopment of new microbial community diagnostics and therapeutics (Borignen et al., 2013). Nowadays, the need of non-invasive biomarkers is considerable and urgent, taking into account that the average delay between the first symptoms and the laparoscopic diagnosis is approximately 7 years. In the present article, the current status of noninvasive diagnostic biomarkers of endometriosis, based on –omics technologies, has been extensively discussed and various limitations of these studies have been reported. Though the search for new biomarkers to be used for diagnosis of endometriosis continues, the underlying pathogenetic mechanisms remain unclear, so studies linking molecular biomarkers with possible etiopathogenetic causes are definitely necessary.

Moreover, by using non-invasive biomarkers the unnecessary laparoscopies will be avoided (Palmer and Barnhart, 2013). The –omics revolution in endometriosis research is ongoing, as proven by the big number of recent publications appeared to date (Saare et al., 2017). Obviously, enthusiasm for the future of –omics in endometriosis must be tempered with a word on costs because these approaches are being used for laboratory and research purposes so far and not for routine clinical use. Data generated by these methodologies, from several patients, would require enormously high number of gigabytes for storage on computer, while experts in bioinformatics are also needed. To overcome these problems, coordinated efforts of many groups are needed that will adapt the methods of multi-scale omics data generation from one specific disease to multiple diseases and will manage an integration between them (Hasin et al., 2017). Results from proteomics analysis are very encouraging and promising for the discovery and establishment routinely of non-invasive biomarkers (Saare et al., 2017). The ultimate vision should be the strict limitation of the symptoms and the improvement of fertility in endometriosis-affected women.

Elucidation of the interactions between different molecular elements and integration of the various levels of –omics using a systems biology approach is promissious to raise novel perspectives on the pathogenic cascades leading to endometriosis. Despite of the substantial amount of data provided to the researchers by the –omics studies, the full potential of this information is not possible to entirely used so far. A major reason may be the operation of all these traditional –omics studies at the level of systems biology and/or multicellular analysis. Thus, the –omics information from single cells or from homogenous cell populations isolated from lesions may offer novel information regarding the true molecular changes appearing to be specific for endometriosis (Saare et al., 2017), thus enhancing the perspectives for a beneficial management of the disease.

Declaration of Competing Interest

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