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The immune pathway through endometriosis to ovarian cancer

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Abstract

Endometriosis is an estrogen-dependent inflammatory disease, defined by the presence of functional endometrial tissue outside of the uterine cavity. This disease is one of the main gynecological diseases, affecting around 10~15% women and girls of reproductive age, being a common gynecologic disorder. Although endometriosis is a benign disease, it shares several characteristics with invasive cancer. studies support that it has been linked with an increased chance of developing endometrial ovarian cancer, representing an earlier stage of neoplastic processes. This is particularly true for women with clear cell carcinoma, low-grade serous carcinoma and endometrioid. However, the carcinogenic pathways between both pathologies remain poorly understood. Current studies suggest a connection between endometriosis and endometriosis-associated ovarian cancers (EAOCs) *via* pathways associated with oxidative stress, inflammation, and hyperestrogenism. This article aims to review current data on the molecular events linked to the development of EAOCs from endometriosis, specifically focusing on the complex relationship between the immune response to endometriosis and cancer, including the molecular mechanisms and their ramifications. Examining recent developments in immunotherapy and their potential to boost the effectiveness of future treatments.

INTRODUCTION

Endometriosis, an estrogen-dependent inflammatory disease, is defined by the presence of functional endometrial tissue (stromal cells and gland) outside of the uterine cavity. It involves ectopic implantation of endometrial cells, marked by heightened proliferation, infiltration, and migration. This condition is one of the main gynecological diseases, affecting around 10~15% women and girls of reproductive age. It can reach 50% of women facing infertility and often correlates with dysmenorrhea and pelvic pain[1,2]. Notably, more than 52% of women diagnosed with endometriosis are between 18-29 years[3].

Regrettably, there is a substantial delay of almost 6 years between the onset of symptoms and diagnosis in primary care, which has a detrimental impact on the quality of life of many women and the subsequent treatment of a large number of patients[4]. Currently, there is no definitive cure for endometriosis, and available treatments primarily focus on symptom management, lacking measures to prevent recurrence of the disease. Risk factors for endometriosis include early menarche, nulliparity, dysfunctional uterine bleeding, aberrant estrogen levels, and low body mass index[5-7].

The underlying mechanisms of this disease have yet to be determined, despite numerous theories attempting to clarify their nature. The most widely accepted theory posits that retrograde menstruation allows endometriotic cells to evade the apoptotic pathway, leading to a disruption of the immune balance in the surrounding endometrioid tissue and triggering an immunological cascade that produces a mixture of pro- and anti-inflammatory factors[8]. However, the precise nature of these alterations remains unclear and is the subject of ongoing research.

Although endometriosis is a benign disease, more and more studies support that it has been linked with an increased chance of developing ovarian cancer, representing an earlier stage of neoplastic processes[9-11]. The first histological correlation between endometriosis tissue and ovarian cancer specimens was first presented by Sampson (1925), proposing that endometrial ovarian cancer may develop from endometriotic

tissue, creating some criteria for diagnosis: (1) evidence of coexisting tumor and endometriosis in the same ovarian location; (2) exclusion of a second malignancy elsewhere; and (3) histological pattern that resembles endometrial origin[12]. Later on, in 1953, Scott added a fourth criteria: (4) histological demonstration of benign lesions of endometriosis adjacent to malignant tissue[13].

Ovarian cancer ranks as the fifth leading cause of cancer-related death among women, surpassing other female reproductive system cancers. Ovarian epithelial tumors are divided into two categories: Type I tumors, which include clear cell carcinoma, low-grade serous carcinoma, endometrioid, and mucinous carcinoma; and Type II tumors, represented by high-grade serous carcinomas[14]. Endometrioid and clear cell tumors are linked to endometriosis and are classified as endometriosis-associated ovarian cancers (EAOCs), exhibiting a crescent correlation as they progress from endometriotic cyst epithelium through various stages of tumor development[15]. Both endometriosis and cancer share certain characteristics, such as the ability to evade apoptosis, form new blood vessels, and metastasize to distant sites, as well as the capability to create a supportive microenvironment that promotes growth and immune system mobilization[11].

Our review focuses on the intricate relationships between the immune response to endometriosis and cancer, particularly on the molecular mechanisms and their consequences. We designed this article by following a chain of thought, starting with the characteristics of the diseases, the immune alterations found in both pathologies, followed by the correlations between endometriosis and ovarian cancer, and how the immune response of endometriosis can lead to the onset of ovarian cancer, or at least favor its development. We explore how the microenvironment and imbalances due to endometriosis can trigger the development of ovarian cancer. Furthermore, we provide an overview of recent advancements in immunotherapy and their potential to enhance the efficacy of future treatments of these pathologies until the new findings for treatment and the current hurdle this field faces.

OVERVIEW ON OVARIAN CANCER THAT CORRELATES WITH ENDOMETRIOSIS:

Despite being a benign disorder, numerous epidemiological studies consistently identify endometriosis as a risk factor for ovarian cancer, which is the most lethal among gynecological malignancies and rank as the third most prevalent[16–18]. Several mechanistic theories are linked to endometriosis, such as the reflux of endometrial tissue through the fallopian tubes during menstruation, coelomic metaplasia, embryonic cell rests, and lymphatic and vascular dissemination[12,19,20]. However, the etiology of endometriosis is generally considered multifactorial due to factors like genetics, hormones, and immunity. Remarkably, endometriosis can also develop in postmenopausal women, and although rare, it carries the risk of malignant transformation[21–24]. Hence, the observation of the occurrence and malignant transformation of endometriotic lesions in a hypoestrogenic environment with the absence of menstrual cycles emphasize the need for a more comprehensive understanding of the underlying mechanisms in the disease's pathogenesis, that goes beyond classical theories centered on estrogen and retrograde menstrual flow[25,26].

The invasive potential of endometriosis and the persistent maintenance of ectopic tissue are characteristics that resemble cancer[27]. Notably, a significant association has been established between a history of endometriosis and an increased risk of developing specific subtypes of epithelial ovarian carcinoma, namely, endometrioid carcinoma, clear cell carcinoma, and low-grade serous tumors[19].

It is estimated that endometriosis increases the risk by approximately 3-fold for endometrioid and clear cell carcinomas[28,29]. Through histopathological analysis, it has been observed that the carcinogenesis of these cancer subtypes can originate from cysts and other endometriotic lesions that progress to a phase of endometriosis with higher oncogenic potential, known as atypical endometriosis[30–32]. In this condition, two main histological findings are noteworthy and may be present simultaneously or independently: cellular atypia (cytologic atypia) and architectural atypia (hyperplasia)[33]. Furthermore, a subsequent prospective histological study found that

endometriosis displaying architectural atypia, and consequently exhibiting higher proliferative activity, is most strongly linked to endometriosis-associated ovarian cancer[34].

From a molecular perspective, atypical endometriosis and clear cell carcinoma share mutations in HNF-1β (hepatocyte nuclear factor-1β) and the ARID1A gene (AT-rich interactive domain-containing protein 1A), which encodes the tumor suppressor protein BAF250. Therefore, absence of the BAF250a protein can be a useful early biomarker indicating malignant transformation of endometriosis[35,36]. In contrast, endometrioid adenocarcinoma primarily exhibits mutations in CTNNB1 (catenin beta 1), PTEN (phosphatase and tensin homolog), and ARID1A[35].

The association between endometriosis and low-grade serous tumors is relatively recent. Previously, epidemiological studies were primarily conducted alongside the high-grade serous tumor subtype, which does not exhibit an association with endometriosis[19]. The two histological subtypes may differ in terms of etiology, with low-grade typically originating from a malignant transformation process of borderline serous tumor, while high-grade tumors often arising from intraepithelial tubal carcinoma, with rarely observed progression from low-grade to high-grade serous tumors[37,38].

Genetically, TP53 mutations are more restricted to high-grade serous carcinomas[39], whereas low-grade serous carcinomas typically exhibit mutations in KRAS (Kirsten Rat Sarcoma) and BRAF (B-type Raf kinase), along with overexpression of hormone receptors (estrogen and/or progesterone)[38]. It's worth noting that KRAS mutations are generally associated with a worse prognosis compared to exclusive BRAF mutations[40]. This underscores the need for additional genetic research in this field.

IMMUNE DYSREGULATION IN ENDOMETRIOSIS AND ESTROGEN DEPENDENCY ON MICROENVIRONMENT DYSRUPTION:

Endometriosis has garnered significant attention in the field of reproductive health due to its elusive etiology. While several theories have been proposed to explain its origin,

the most widely accepted among them is Sampson's theory of retrograde menstruation[41]. According to this theory, during menstruation, endometrial cells retrograde into the fallopian tubes and subsequently into the peritoneal cavity[42,43]. In healthy females, these displaced cells typically undergo programmed cell death and are efficiently cleared by phagocytes and Natural Killer (NK) cells—a phenomenon known as immune surveillance[44,45]. However, in the context of endometriosis, compromised cell-mediated immune responses can disrupt this natural clearance process. Consequently, eutopic endometrial cells can adhere to the peritoneal wall, where they proliferate and eventually form endometriotic lesions[45,46].

Immune surveillance evasion: dysregulated apoptosis signaling

The precise mechanisms responsible for the evasion of immunosurveillance by ectopic endometrial cells (EECs) remain poorly understood. Several hypotheses have been proposed to elucidate this phenomenon, including the possible dysregulation of programmed cell death pathways[47]. The FAS (CD95) and FAS ligand (FASL) extrinsic apoptosis signaling system, which is well-documented for its significant role in immune modulation[48–50], appears to hold a pivotal position in the context of endometriosis[51,52]. In response to the peritoneal microenvironment, specially elevated interleukin-8 (IL-8) levels, a conspicuous increase in the expression of FASL is detected within EECs[53–55]. This heightened FASL expression seems to initiate apoptotic processes through the FAS-mediated pathway in immune cells expressing FAS, including T cells and NK cells[55].

In a parallel manner, alterations in tumor necrosis factor (TNF)-α-mediated cell death signaling contribute to the advancement of endometriosis. In retrograde menstruation, the entry of menstrual tissues into the peritoneal cavity stimulates macrophages to release cytotoxic cytokines, including TNF-α, thereby initiating apoptosis signaling in the extrauterine endometrial fragments that need to be eliminated[56]. However, in individuals with endometriosis, estrogen-dependent molecular alterations in retrograde menstrual tissues enable them to evade TNF-α-mediated apoptosis[57]. In their pioneering research, Han *et al.*[58] presented compelling evidence showcasing elevated

expression levels of Estrogen Receptor β (ERβ) within endometriotic tissues. Erβ exerts regulatory influence over cellular apoptotic processes by impeding apoptosis initiated by TNF-α. Furthermore, ERβ engages in interactions with cytoplasmic inflammasome constituents, consequently stimulating heightened production of interleukin-1β (IL-1β)[58]. This augmentation, in turn, amplifies cellular adhesion and proliferation characteristics.

Concurrently, within endometriotic lesions, there is a notable increase in the abundance of the nuclear receptor coactivator 1 (NCOA1) isoform[59]. NCOA1, also known as Steroid Receptor Coactivator 1 (SRC-1), plays a pivotal role in the regulation of gene expression in response to hormonal signals[60–62] functioning as a coactivator for nuclear receptors, which are transcription factors related with physiological processes, including cell growth, differentiation, and metabolism. In endometriosis, within these specific lesions, the NCOA-1 isoform engages in an interaction with caspase 8, thereby impeding the TNF-α-triggered apoptosis process through disruption of the assembly of apoptosis complex II[59]. Altogether, it's possible to perceive that those alterations contribute to the complex mechanisms underlying the development and persistence of endometriosis. Figure 1 depicts a simplified schematic illustrating how ectopic endometrial cells evade immune surveillance.

Within the framework of endometriosis, perturbation of the intrinsic apoptotic pathway carries substantial ramifications. The B-cell lymphoma/Leukemia-2 gene (Bcl-2) represents a novel class of proto-oncogenes characterized by their ability to inhibit apoptosis independently of cell proliferation stimulation[63,64]. In the context of endometriosis, an upregulation of Bcl-2 protein expression was observed in the proliferative eutopic endometrial tissue of affected patients[65,66]. Conversely, Bax expression was notably absent in the proliferative endometrial phase, but displayed increased expression during the secretory phase in both patients and control subjects[66]. Accordingly, research findings demonstrate that the utilization of Gonadotropin Releasing Hormones analogs elicits an upregulation in the expression of the proapoptotic protein Bax[67], a putative antagonist protein, concomitant with a

downregulation in the expression of the antiapoptotic protein Bcl-2 which can suggest potential targets for therapeutic interventions in this condition.

Dysregulation of the mitogen-activated protein kinase (MAPK) signaling pathway also seems to exert a significant influence on the advancement of the disease[68]. In tandem with the conveyance of antiapoptotic signals to endometriotic tissues, this intricate cascade promotes the recruitment of immune cells, intensifies the inflammatory response, and augments the expression of growth factors[69,70]. This coordinated synchronization of cellular processes seems to promote the initiation and progression of endometriotic lesions, while concurrently fostering a microenvironment conducive to the development of endometriosis.

Macrophages and their influence

Increasing evidence suggests that the peritoneal fluid features macrophages as the most predominant immune cells, within physiological parameters, and that they may be related to the pathogenesis of endometriosis[71]. The ectopic growth of endometriotic tissue within the peritoneal cavity leads to the onset of an inflammatory response, which, in turn, results in increased recruitment of these cells, a phenomenon mediated by colony stimulating factor-1 (CSF-1), monocyte chemoattractant protein-1 (MCP-1/CCL2), interleukin (IL)-8, and RANTES (CCL5)[72-74].

Upon recruitment, macrophages undergo activation, thus adopting specific functional profiles (M1/M2) that can either intensify inflammatory processes or contribute to tissue repair and immune regulation. In this sense, the polarization of macrophages into the M2 phenotype seems to be beneficial to the angiogenesis of endometriotic lesions, through secretion of vascular endothelial growth factor (VEGF)[75]. Additionally, the secretion of factors such as IL-10 and transforming growth factor-beta (TGF-β) by M2-polarized macrophages contributes to the growth of endometriotic lesions, since it impairs the cytotoxicity of natural killer (NK) cells[76-78]. Interestingly, there is also growing interest in the role of IL-17A in endometriosis, as it appears to be associated with macrophage recruitment, triggering M2 phenotype polarization, angiogenesis and maintenance of the inflammatory cascade[79,80].

In addition to the intricate interplay involving macrophages and the aforementioned mechanisms, it is essential to address the role of fibrosis in this context. In this sense, TGF-β secreted by M2 macrophages induces fibrosis, as it promotes the differentiation of fibroblasts into myofibroblasts and stimulate the synthesis of collagen and fibronectin[81–83]. On the other hand, Barcz *et al.* suggests that increased levels of VEGF are negatively associated to endometriosis-related pelvic fibrotic adhesions[84].

Macrophages also play a role in neurogenesis and, consequently, in the onset of endometriosis-associated pain[85]. Overall, nerve fibers originating from endometriotic lesions have the capacity to secrete chemokines such as CCL2 and CSF-1, which, as previously discussed, are pivotal in the recruitment of macrophages[81,86]. As a result, macrophages release neurotrophic factors, including brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), thus contributing to the heightened sensitivity and pain experienced by individuals with endometriosis[86].

Furthermore, iron accumulation into the peritoneal cavity, stemming from retrograde menstruation, holds a particular interplay with macrophages and plays a role in the pathogenesis of endometriosis[87]. Macrophages in the pelvic cavity carry out erythrocyte phagocytosis and iron metabolism, thereby resulting in elevated iron concentrations within the peritoneal fluid[88]. As a result, iron overload can trigger oxidative stress and contribute to chronic inflammation, thus leading to increased proliferative capacity of endometriotic lesions[89].

Finally, it is crucial to acknowledge that the phenotypic distinction between M1 and M2 macrophages is currently viewed as oversimplified[87]. Indeed, emerging evidence underscores the plasticity of macrophages, suggesting the potential for a hybrid M1/M2 profile or a dynamic switch between these phenotypes[87,88]. This adaptability appears to be influenced by the specific microenvironment to which these cells are exposed[88]. Such complexity in the interplay between macrophages and the pathogenesis of endometriosis emphasizes the need for further studies to thoroughly elucidate these intricate mechanisms.

Natural Killer cells dysfunction

Natural Killer cells play a crucial role in the immune system's surveillance and defense against endometriosis. These immune cells are capable of identifying and eliminating abnormal endometrial cells, thereby contributing to the body's efforts to combat this condition[90,91]. Additionally, NK cells help regulate inflammation, modulate angiogenesis, and assist in maintaining immune tolerance within the endometrial environment[92,93].

In individuals with endometriosis, the peripheral circulation is characterized by a predominance of CD16$^+$/CD56^{dim} NK cells, which are well-known for their heightened cytotoxic capabilities. In contrast, the endometrium and peritoneal fluid (PF) predominantly harbor CD16$^-$/CD56^{bright} NK cells, renowned for their robust production of cytokines[94]. Accordingly, among females diagnosed with endometriosis, there is a noteworthy decrease in cytotoxicity observed in NK cells present within the peritoneum and PF[95]. Reduced cytotoxicity in the context of endometriosis may result from a complex interplay of cytokines within the intricate microenvironment of endometriotic lesions.

For instance, Yang et al.[76] recently proposed that the interaction between macrophages and endometrial stromal cells (ESCs) could downregulate NK cell cytotoxicity. This downregulation might occur through the induction of cytokine secretion, including IL-10 and TGF-β, by the interacting cells. Such an interaction could potentially facilitate immune evasion by ectopic fragments and contribute to the development of endometriosis. Building on this idea, Kang et al.[96] demonstrated that an increased level of IL-6 in the PF of patients with endometriosis might also suppress NK cell activity via regulation of SHP-2 expression. Likewise, elevated IL-15 Levels were demonstrated to foster the proliferation and invasive behavior of ESCs while concurrently suppressing the cytotoxic capabilities of NK cells in individuals with endometriosis[97]. These cytokines, each wielding a distinct mechanism, collectively contribute to the precise regulation of NK cell responses in various immunological contexts.

The NK cell detection system employs a set of receptors on the surface of NK cells, including activating receptors like NKG2D and CD16 (FcgRIIIa), to regulate NK cell

activities. This system is crucial for the immune system's ability to identify and eliminate abnormal cells—such as ECCs. In comparison to healthy women, the PF of individuals with endometriosis exhibited decreased levels of various markers associated with NK cell cytotoxicity. These markers include the natural receptors NKp46, NKp44, and NKG2D, as well as CD16 and CD107a, which are indicative of NK cell activation, and CD69[98].

Conversely, Gonzalez-Foruria *et al.*[99] demonstrated that there is a notable rise in soluble NKG2D ligands in the PF of endometriosis patients, indicating reduced expression of these ligands on the surface of ectopic endometrial cells[99]. These soluble NKG2D ligands serve as decoy receptors, contributing to increased evasion from NK cell recognition[100]. Notably, several studies have also reported elevated levels of Inhibitory Receptor Tyrosine-based Inhibition Motif-Killer Immunoglobulin-like Receptors (ITIM-KIRs), including KIR2DL1, Natural Killer Cell Inhibitory Receptor NKB1, EB6, soluble intracellular adhesion molecule-1 (I-CAM), and Human Leukocyte Antigen class I (HLA-I) in the PF of endometriosis patients[101–104].

Thus, the immune dysregulation associated with endometriosis involves intricate interactions between NK cells, various immune cells, and cytokines, ultimately impacting NK cell function and contributing to the development and persistence of this condition.

Altered T-cell-mediated cytokine profiling and cytotoxicity in endometriosis

In the context of endometriosis, there is a notable reduction in the Th1/Th2 cell ratio within the peritoneal fluid (PF) when compared to women with a healthy condition[105]. This shift is accompanied by increased concentrations of IFN-γ and IL-10, resulting in elevated IL-4/IFN-γ, IL-4/IL-2, IL-10/IFN-γ, and IL-10/IL-2 ratios within endometriotic lesions[106]. Furthermore, individuals with endometriosis show a substantial reduction in the T-bet/GATA-3 protein ratio compared to their healthy counterparts[107].

To our current understanding, T-bet regulates the expression of the Th1-specific cytokine IFN-γ while inhibiting the production of the Th2-specific cytokine IL-4[108–110].

Conversely, GATA-3, a transcription factor specific to Th2 differentiation, orchestrates the differentiation of Th2 cells and promotes the production of Th2 cytokines, including IL-4, IL-6, and IL-10[111–113]. Within endometriotic lesions, there is a significant upregulation of GATA-3 protein mRNA levels influenced by estrogen, a hormone central to GATA-3 regulation[107]. Consequently, the interplay between GATA-3 and estrogen signaling governs the production of Th2-type cytokines in affected endometrial cells[114]. This dynamic contributes to the elevated levels of Th2-type cytokines in endometriotic lesions—despite the increase in IFN-γ concentrations—ultimately promoting the progression of endometriosis.

Recent research conducted by Xia et al.[115] underscores the diagnostic significance of serum cytokine concentrations in the context of endometriosis-associated pelvic pain (EAPP). Specifically, the study identifies IFN-γ and IL-2 as independent protective factors against EAPP, while recognizing IL-4 and IL-10 as independent risk factors for the condition[115]. Notably, IL-4, a hallmark cytokine associated with the Th2 immune response, is shown to elevate localized estrogen levels, thereby facilitating the estrogen-dependent progression of endometriosis[116]. Furthermore, this cytokine enhances the proliferation of endometriotic stromal cells through the activation of pathways such as p38 MAPK, stress-activated protein kinase/c-Jun kinase, and p42/44 MAPK, thereby leading to the advancement of the disease[117].

Apart from Th2 cells, T-helper-17 (Th17) cells and Regulatory T (Treg) cells may also be involved in endometriosis[118]. Khan et al.[118] recently demonstrated that CD4+IL-17A+ Th17 cell percentage was consistently reduced in both peripheral blood and PF of individuals with early and advanced endometriosis. In contrast, Gogacz and colleagues reported an elevated proportion of Th17 cells in PF when compared to peripheral blood in individuals with endometriosis[119]. Their findings further indicated that the percentage of Th17 cells in PF was associated with the severity of endometriosis[119].

Zhang and colleagues[120] pioneered the empirical validation of elevated IL-17 Levels in the PF of individuals with endometriosis. Their research provided substantial evidence of statistically significant increases in IL-17 concentrations in individuals with

minimal/mild endometriosis compared to those with moderate/severe disease and healthy individuals[120]. Subsequent to their groundbreaking work, multiple other authors have also confirmed elevated IL-17 Levels in the PF of women diagnosed with this condition[121].

Interleukin-17A exhibits the capability to induce the secretion of IL-8 and the upregulation of cyclooxygenase-2 (COX-2) expression, thereby instigating inflammatory reactions and fostering the proliferation of stromal cells associated with endometriosis[122]. In a similar vein, the research conducted by Ahn et al.[79] has provided evidence that IL-17A also contributes to the pathogenesis of endometriosis by triggering the expression of angiogenic factors such as vascular endothelial growth factor (VEGF) and IL-8, as well as proinflammatory cytokines including IL-6 and IL-1β, along with chemotactic cytokines such as granulocyte colony-stimulating factor (G-CSF), C-X-C motif chemokine ligand 12 (CXCL12), C-X-C motif chemokine ligand 1 (CXCL1), and C-X3-C motif chemokine ligand 1 (CX3CL1)[79].

Subsequently, it was also observed that the presence of IL-10+Th17 cells significantly rises in the PF of females suffering from endometriosis[123]. Additionally, there is an upregulation of IL-27, IL-6, and TGF-β in this context. In comparison to peripheral CD4+ T cells, endometrial CD4+ T cells exhibit a pronounced expression of IL-27 receptors, particularly in the ectopic endometrium. Apparently, in later stages endometriosis, IL-27 seems to plays a role in suppressing the development of Th17 cells while stimulating the production of IL-10 within these cells through the c-Maf/RORC/Blimp-1 complex—thereby contributing to the establishment of an immune tolerance pattern[123]. Consequently, these Th17 cells, which produce IL-10, enhance the growth, adhesion, invasion, and deep infiltration of endometrial stromal cells, thereby hastening the progression of endometriosis[123,124].

In contrast to the CD4+IL-17A+Th17 cell subset, there is a substantial increase in the proportions of CD25+FOXP3+ Treg cells within the CD4+ T-cell population among patients with advanced endometriosis, as opposed to those with early-stage endometriosis or control subjects (P < 0.05 in both instances)[118]. The induction of Treg

cells, characterized by the expression of the transcription factor FOXP3, may be facilitated by specific cytokines, notably TGF-β and IL-10[125]. Consistent with these findings, heightened levels of TGF-β and IL-10 have been consistently documented in the PF of individuals afflicted with endometriosis[90,126]. Notwithstanding this, endometriosis is correlated with elevated PF concentrations of numerous cytokines, encompassing various chemotactic and activatory factors, such as RANTES and MCP-1, known as robust chemoattractants for Treg cells[127,128]. Hence, the heightened prevalence of the CD4+ T cell phenotype may arise from either local stimulation or represent a secondary occurrence associated with their chemotactic response due to the sustained presence of a local inflammatory response[118].

It is hypothesized that the abundance of Treg cells within the peritoneal cavity hinders the recognition and selective targeting of ectopic endometrial tissues—thereby contributing to the persistence of ectopic lesions.

B cell dysregulation in endometriosis

Notably, the role of B cells in endometriosis is an area of ongoing research, and the exact mechanisms underlying their dysregulation in the disease are not fully understood. One intriguing finding is the decreased B-cell leukemia lymphoma (Bcl)-6 and increased B lymphocyte inducer of maturation program (Blimp)-1—transcription factors that regulate B-cell function—in the peritoneal cavity of patients with endometriosis[129].

Blimp-1 serves as a pivotal regulator of plasma cell differentiation[130,131]. The pronounced elevation of Blimp-1 in individuals suffering from endometriosis implies a heightened commitment to the differentiation of B cells into plasma cells. This observation raises the intriguing possibility of an intensified antibody response occurring within the peritoneal cavities of these patients, potentially bearing significance for their immune function. Conversely, Bcl-6 functions as an antagonist to Blimp-1, primarily inhibiting the process of plasma cell differentiation[132]. The diminished levels of Bcl-6 in endometriosis patients suggest a compromised ability to regulate the differentiation of B cells into plasma cells. This imbalance may contribute to

an exaggerated antibody response or potentially exert influence over other facets of immune function.

The PF of individuals with endometriosis also seems to express high levels of the B lymphocyte stimulator (BLys)[133]—a protein that plays a critical role in the development of B cells and their differentiation into plasma cells[134]. Increased BLys levels in endometriotic lesions, in turn, suggest that the local microenvironment within the peritoneal cavity of endometriosis patients may be conducive to enhanced B-cell activation and maturation.

Accordingly, the presence of autoantibody responses targeting endometrial antigens represents a prevalent characteristic in endometriosis. In 1980, Startseva[135] was the first to report an elevated responsiveness of B cells in individuals with endometriosis. Since then, antibody responses directed against a range of both serum and tissue antigens, including alpha(2)-Heremans Schmidt glycoprotein (alpha(2)-HSG), transferrin, and carbonic anhydrase, have been discerned in this condition[136]. Nevertheless, additional research is required to gain a more comprehensive understanding of the connection between autoantibodies and the disease's onset and progression.

Estrogen and the immune microenvironment in endometriosis

The development of endometriotic lesions relies on estradiol—an estrogenic steroid hormone[137]. The heightened activity within the 17β-estradiol axis serves as a pivotal trigger for the activation of macrophages intricately associated with endometriosis pathogenesis[138-141]. In response to the escalated signaling of estradiol, the ectopic endometrial tissue, a hallmark feature of endometriosis, undergoes a noteworthy upregulation in the expression of estrogen receptor beta (ERβ). Indeed, higher ERβ levels, as opposed to ERα, have been observed in endometriotic tissues when compared to normal endometrial tissues[142]. An elevated ERβ-to-ERα ratio within endometriotic stromal cells is linked to the downregulation of progesterone receptors and an upsurge in cyclo-oxygenase-2 Levels, thereby playing a role in the development of progesterone resistance and inflammation[143,144].

Moreover, elevated prostaglandin levels hinder the immune system, enabling ectopic endometrial cells to evade immune surveillance and form endometriotic lesions. Additionally, ERβ engages in interactions with cytoplasmic inflammasome components and TNF-α-mediated programmed cell death pathways, resulting in increased production of IL-1β and enhanced cellular adhesion and proliferation[58]. This intricate modulation ultimately creates a cellular environment favoring enhanced cell survival and the sustained orchestration of the inflammatory response, both of which are pivotal factors in the perpetuation of endometriosis.

IMMUNE FACTORS DRIVING OVARIAN CANCER:

The correlation between chronic inflammation and development of tumors is not a unique feature of ovarian cancer. It has been described for many years, as various risk factors of cancer development are linked to inflammatory processes, such as viral infections, smoking and UV exposure[145]. The process of ovarian carcinogenesis is attributed to multiple factors, and while inflammation does not account for all of them, it serves as a pivotal element in the development of this particular disease[146]. Firstly, despite ovulation being a physiological process, multiple factors that alter the ovulation cycle, such as contraceptive pills, parity and age of menarche and menopause are related to a reduced risk of ovarian cancer development[147]. Fathalla proposed, in 1971, the theory of incessant ovulation, suggesting that the repetitive damage and subsequent repair of the ovarian epithelium may elevate the risk of neoplastic development and be the reason for the above-mentioned risk factors[147].

Presently, it is well-established that ovulation is closely connected to the inflammatory cascade, as the ovarian population of immune cells play critical roles in various processes within the menstrual cycle. As an example, ovarian macrophages contribute to tissue repair and proliferation through the secretion of several growth factors, TGF-β and IL-10, as well as apoptosis *via* the secretion of Reactive Oxygen Species (ROS), and IL-1β during physiological destruction, resulting in the necessary rupture of the follicle

wall for ovum liberation and remodeling processes in the ovarian epithelium associated with the menstrual cycle phases[148].

The result of this is a chronic and periodic exposition of ovarian epithelial cells to a complex and dysregulated interplay of molecular events, involving both inflammation and tissue proliferation stimuli. These events encompass the nuclear factor-kappa B (NF-κB) activation, which has been reported to be an important element in tumorigenesis and further fueling the inflammatory milieu, as it enhances cytokine and growth factors production, induces cell proliferation and impedes cell apoptosis[149–151]. It is also important to note that the high levels of ROS may induce DNA damage that can facilitate the development of mutations that could induce the ovarian carcinogenesis process[152]. Ultimately, the complicated network of interacting events offers insights into its plausible involvement in instigating the mechanisms underpinning ovarian carcinogenesis.

Furthermore, various inflammatory conditions, including infections and reproductive system disorders, have been identified as risk factors for the development of ovarian cancer. Lin and colleagues, in 2011, found that women with Pelvic Inflammatory Disease (PID) exhibited an adjusted Hazard Ratio for ovarian tumor development almost twice as high as non-affected women[145]. Additionally, in 2012, a combination of results from 13 case-control studies demonstrated a significant association between the presence of clear-cell, low-grade serous, and endometrioid invasive ovarian cancers and a history of endometriosis among patients[19].

A determining factor in how ovarian cancer will progress, is the individual aspects of the tumor microenvironment. Increased number of Tumor infiltrating lymphocytes (TILs) with active CD3+ T cells have been associated with increased survival rate in patients with ovarian cancer[153,154]. On the other hand, the anti-tumor action of these cells can be rendered less effective by Tumor-infiltrating immune cells with immunosuppressive activity, such as M2 macrophages, regulatory T cells (Tregs) and Myeloid-derived Suppressive Cells (MDSCs), whose increased presence have been consistently related with poor prognosis of the disease[155,156].

Previous studies have evaluated the specific action of live Treg cells in this scenario. Initially, production of CCL22 chemokine by cancerous cells and macrophages attracts CCR4 expressing Treg cells to tumor site. After reaching the tumor microenvironment, these cells highly express several immunosuppressive cytokines, such as IL-10, IL-35, and TGF-β[157,158] and immune checkpoint inhibitors (CPI), such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), that binds to CD80/CD86 in Antigen presenting cells (APC), decreasing co-stimulation of T cells and inhibiting their activity[157]. In summary, the interplay among all of these factors underscores a multifaceted tumor microenvironment that hinders immune surveillance and the fight against malignant cells.

Another important molecule for the immunomodulator properties of ovarian cancer microenvironment is T cell immunoglobulin and mucin domain-containing protein 3 (TIM3), associated with higher IL-10 production and inhibition of T cell action in multiple tumors[158]. Currently, it is being tested in preclinical settings as a possible target for monoclonal antibodies in cancer treatment in association with PD-1/PD-L1 inhibitors[159,160].

It is important to note that proinflammatory cytokines, such as IL-6 and TNF-α, can also exhibit high expression levels within the context of ovarian cancer[161]. The correlation between IL-6 and the tumor's progression is well-established, and it is believed to contribute to various functions, primarily through the JAK/STAT pathway, including angiogenesis, cell proliferation, differentiation, and resistance to chemotherapy[162]. This could explain why the overexpression of the IL-6 receptor (IL-6R) in ovarian tissue has been linked to a poor prognosis for the disease[163].

Regarding M2 macrophages, in addition to playing an immunosuppressive role, they can induce angiogenesis and fibroblast proliferation by secreting various growth factors that promote the replication of cancer cells[164]. This is particularly significant in the clinical context of ovarian cancer, as vasculogenesis driven by VEGF secretion is the primary target of bevacizumab, one of the immunotherapy drugs currently used in the treatment of this condition[165].

Another type of CPI is PD-1, whose ligands PD-L1 and PD-L2 (both members of B7 superfamily) can be expressed by both immune and cancerous cells in the tumor microenvironment. When the PD-1 receptor of T cells binds to its ligands, it causes a reduction in proliferation, cell activity, IFN-γ and IL-2 production, and may even induce apoptosis of these cells[157,166,167]. Given the pressing need for additional therapeutic targets to enhance outcomes in combined ovarian cancer treatment, current research focuses on investigating the potential roles of other B7 family members, specifically B7-H3 (CD276) and B7-H4 (B7x) in TME immune suppression, as their expression relate to poor survival rates and treatment resistance[168–170].

Research suggests that B7-H3 and B7-H4 exhibit distinct expression patterns within the ovarian cancer microenvironment, as B7-H3 has been shown to be present both in stromal and tumor cells in Epithelial Ovarian Cancer (EOC) TME, whereas B7-H4 seems to be primarily restricted to tumor cells within the ovarian cancer[171]. Furthermore, the expression of B7-H4 by Tumor-Associated Macrophages is induced by IL-6 and IL-10 and B7-H4 expressing TAMs in the ovarian cancer microenvironment exhibit an enhanced suppressive effect on T cell responses[172]. B7-H4 is believed to trigger cell cycle arrest in T lymphocytes, leading to the inhibition of cell division and proliferation.

Additionally, it has been linked to reduced cytokine production and a decrease in the cytotoxic capabilities of these immune cells[173]. On the other hand, B7-H3 has a complex and conflicting role in the immune response, as it may function as both a co-stimulatory and immunoregulatory molecule. This dual role could explain why a study discovered a positive correlation between B7-H3 expression, CD8+ cell infiltration in the TME, and improved prognosis for patients with pancreatic cancer[174]. However, in most neoplasms, including ovarian cancer, high B7-H3 expression doesn't appear to be associated with a favorable prognosis. Instead, it is correlated with increased therapy resistance and enhanced proliferation of cancerous cells, both *in vitro* and in vivo[170].

B7-H3 may be expressed in Antigen-Presenting Cells, and the knockout of its expression has been shown to induce enhanced cytolytic activity in tumor antigen-

specific CD8+ cells among Tumor-Infiltrating Lymphocytes of mice, resulting from a higher production of IFN-γ and Granzyme B enzymes[175]. It is important to note, however, that the precise mechanisms and functions of B7-H3 and B7-H4 in immune cell activity are still areas of active research. Despite this, these proteins offer potential avenues for future tailored immunotherapies that can improve outcomes in ovarian cancer treatment.

ENDOMETRIOSIS AND OVARIAN CANCER: WHAT IS KNOW UNTIL NOW?

The presence of endometriosis leads to an increased risk of malignant tumors, and it is a well-documented finding that both pathologies appear together[176]. Suggesting some kind of transformation from endometriosis constituents into tumor cells[177]. Current molecular studies aim to establish links between endometriosis and EAOCs through pathways related to oxidative stress, inflammation, and hyperestrogenism[178]. Several researches have indicated that atypical endometriosis precedes clear cell or endometrioid ovarian cancers[31,32,179–181], suggesting a precancerous behavior[182]. A study conducted by Kato et al. concluded that certain epithelial cells in ovarian endometriosis, including atypical endometriosis and endometriosis - with many inflammatory and regenerative changes - have already acquired a clear cell phenotype[183].

Thinking about the pathway through endometriosis to derived ovarian cancer, the first thing to point out is the common component of both pathologies, the inflammatory pattern and immune system mobilization[11]. Those alterations can lead to a disruption and tumor formation. Endometriosis patients may have an inflammation profile similar to those with EAOC, even present in patients with benign lesions[184], suggesting that tumor–like immune signatures may develop even earlier than imagined. Deep infiltrating endometriosis with infrequent occurrences exhibits a tumor-like behavior, and may even resemble a metastatic disease[185,186].

Suryawanshi et al., in 2014, demonstrated a correlation between upregulation of the complement pathway and the KRAS and PTEN-regulated pathways, those two

frequently related in oncogenesis and maintenance of the cancer phenotype in vitro. Also, this study showed that 33% of the patients with endometriosis revealed a tumor-like inflammation[184]. Complement was previously linked with the support of tumor growth, being engaged in both chronic and acute inflammation[187,188].

It is crucial to comprehend the onset of the lesion and its connection to the inflammatory component. The development of endometriosis initiates with the implantation of ectopic tissue, which leads to bleeding. This is followed by inflammation, which triggers fibrin deposition and adhesion formation, eventually leading to scarring and distortion of the affected surfaces[189]. It has been reported that eutopic endometrium has a significant decrease in apoptosis compared to women without endometriosis[190]. The inflammatory process of endometriosis is strongly correlated with the peritoneal space experiencing high levels of oxidative stress, this leads to the proliferation of endometriosis as well as increased angiogenesis[191].

Increased proliferation of endometrial tissue and the occurrence of retrograde menstruation result in elevated levels of hemoglobin, heme, and iron[192,193]. Intense hemolysis observed in endometriosis results in high levels of free heme and iron; these molecules have a prominent effect as proinflammatory factors[193]. Excessive exposure to iron in the context of endometriosis sustains a state of chronic inflammation, modulates several mechanisms for the progression of endometrial lesions, and generates intracellular reactive oxygen species, as well as activating neutrophil responses[194,195].

These substances modify crucial structures, enhance adhesion of refluxed endometrial cells, result in cell damage and DNA methylation, and consequently lead to the development of fibrosis and progression of endometriosis[189,193,196]. Subsequent transcription activation occurs (NF-kB, AP-1, and SP-1), along with oxidative burst, production of ROS, and IL-8[197-199]. Iron overload worsens the activation of peritoneal macrophages. And help to maintain a state of chronic inflammation. The inflammatory is further accentuated by the increased expression and activity of COX- 2, interleukins,

and oxidative stress that act through the mitogen-activated protein kinase (MAPK) pathways[190].

The peritoneal fluid in cases of endometriosis typically has elevated levels of activated cytokines and macrophages[200,201]. Macrophage activation is implicated in the pathogenesis of endometriosis and its association with ovarian cancer. Primarily due to the trophic factors secreted by macrophages that promote the growth of neoplastic lesions while at the same time increasing the conditions of oxidative stress due to the production of lipid peroxides[202,203]. Macrophage proliferation can alter the immune response at the site of inflammation, M2 phenotype molecules attract additional proinflammatory mediators to the lesion site, amplifying the inflammatory microenvironment[204].

Tumor-associated macrophages (TAMS) are a key component of the tumor stroma, essential for angiogenesis and matrix remodeling[189], they spontaneously release large amounts of IL-10 to TGF47, and some chemokines induce IL-10 in macrophages and the monocyte chemotactic protein-1 polarizes immunity in the Th2 direction[205,206].

Other than that, macrophages secrete many products such as TGF-beta, VEGF, IL-1, PGE2 and macrophage migration inhibitory factor (MIF)[35]. Importantly, MIF sustains macrophage viability, which sustains inflammation through TAMS activation, and leads to tumor progression and the development of metastases[207]. Also, MIF upregulates COX-2 synthesis and Prostaglandin E2 (PGE2) secretion in ectopic endometrial cells[208].

In a synergistic manner, IL-1 is associated with the induction of cyclooxygenase-2 (COX-2) and interleukin-8 (IL-8) expression, which facilitate migration, proliferation, and angiogenesis in endometriotic tissue[209]. And as a cytokine that promotes tumor growth, IL-1 triggers an ongoing chemical dialogue between the progressing tumor and its supportive stroma[210]. In the ovary, COX-2 is involved in the early events of neoplastic transformation; it is rarely found in normal ovarian epithelium, but is present in endometriosis and in ovarian cysts considered to be premalignant[211].

In addition, other cytokines and chemokines are present in increased concentrations, on both ovarian CA and endometriosis. These include TNF-α, IL-1β, IL-6, IL-8 and RANTES. The proinflammatory cytokines TNF-α and IL-1β are elevated in the peritoneal fluid of women with endometriosis[201]. IL-1β induces expression of RANTES more in endometriotic stromal cells than in normal endometrial stromal cells, functioning as a chemoattractant for monocytes, memory T cells, and eosinophils[193,201]. TNF also can be found in malignant and/or stromal cells in human ovarian and the expression of TNF-α is increased on clear cell ovarian carcinoma, when compared with normal ovarian tissue[205,211]. Elevated TNF network gene expression resulted in increased signaling related to angiogenesis, cell adhesion, cell cycle and inflammation[211]. IL-8 increases ERα activity to induce ovarian cancer cell proliferation, it acts as an autocrine growth factor to promote proliferation of endometrial stromal cells in normal endometrioma and endometriotic cells.

Endometrial fragments are able to adhere to surfaces due to the presence of molecules that regulate cell-matrix and cell-cell interactions expressed by endometrial cells. These molecules include cadherins, integrins, proteoglycans such as the immunoglobulin superfamily, and CD44[212]. Cell–cell and cell–matrix adhesion molecules are engaged both in cancer tumors and endometriosis[213]. Endometriosis is reported to have an overall highly variable and aberrant integrin expression as compared with eutopic endometrium[190,214–216]. Endometrioid tissue may share molecular mechanisms of invasion and metastasis with carcinoma cells that are related to the level of E-cadherin expression[213,216].

The failure to remove fragments of menstrual effluent from the abdominal cavity induces excessive local inflammation[217,218]. The chronic aberrant expression of proinflammatory cytokines alters regulatory signaling pathways, which may facilitate cancer growth, invasion and metastasis through DNA damage and inhibition of DNA repair *via* reactive oxygen, autocrine/paracrine growth, survival factors for malignant cells, induction of vascular permeability and extravasation of fibrin/fibronectin[205]. And, result in accumulation of genetic mutations in endometriotic cells, through the

changing of physiological homeostasis and progressive transcriptional changes can drive sustained proliferation and increase the rate of DNA repair[189].

Another factor that has an imperative role on disruption of homeostasis, and inflammation, is the hormonal component. As previously mentioned, hormonal management, upregulation of estrogen and intolerance to progesterone, plays a fundamental role in the maintenance and development of endometriosis, as well as on the tumor development. This said, an important alteration is the estrogen role on endometriosis, and its intense presence on peritoneum of afflicted women, and the consequently exacerbation of the immune inflammatory pathway.

Endometriotic stromal cells contain numerous specific epigenetic defects that favor overproduction of E2 and overexpression of the steroid receptor ER-beta that mediates an intense and E2-induced inflammatory process involving overproduction of cytokines and prostaglandins[219-221]. Being a major regulator of all key pathological processes in endometriosis and enhances lesion survival and inflammation leading to pain[220]. And excess E2 can result in cellular proliferation through the stimulation of cytokine production, specifically IL-8 and RANTES[222]. In addition, E2 stimulates the production of PGE2, the micro-environment in endometriotic tissue is marked by proliferative pressure with an enhanced level of reparative activity and thus, a higher chance for DNA damage and mutations[178]. Thus, PGE2 is a central mediator of the inflammatory response on endometriosis, but it has also been shown to regulate vital processes related to tumor growth, including angiogenesis, proliferation and inhibition of apoptosis[223].

Moreover, massive concentrations of estrogen in the ovary may also stimulate the inflammatory process *via* ER-beta in endometriotic stromal cells, which may contribute to the carcinogenic process in neighboring epithelial cells[58,224]. Is speculated that intense inflammation, progesterone resistance, and high levels of E2 (unopposed by progesterone action) in the stromal component led to a high proliferative activity and enrichment of driver mutations (e.g., PIK3CA, KRAS, ARID1A) in attached endometriotic epithelial cells. [68]. And it is associated with pro-inflammatory cytokines,

which leads to (VEGF) expression, cell cycle activation, and activation of the anti-apoptotic gene Bcl-2[225,226]. Figure 2 depicts a simplified schematic illustrating of general immune response on endometriosis and ovarian cancer, and their similarities.

The dysregulation of apoptotic pathways and subsequent resistance to apoptosis contribute to the failure of immune clearance[45,227]. Accumulation of mutations in tumor suppressor genes and oncogenes is a crucial step during tumor development[228]. It is known that hormonal dysregulation in endometriotic implants, along with Inflammatory responses, may drive carcinogenesis [71]. Mutations secondary to endometriosis, is a fair finding. A study conducted by Koppolu *et al.,* in 2021, showed that all the patients with endometriosis recruited on the study had no history or features of neoplastic disease, however the results revealed mutations in known cancer driver genes, especially in ectopic lesions[185]. Some cancer-related mutations are found in endometriosis without cancer, in particular recurrent KRAS mutations[229]. Otherwise, some studies have shown confirmatory evidence that mutations found in endometriosis-associated cancers are found in adjacent endometriosis[36,230–232], and has been reported to exhibit a high percentage of PIK3CA and KRAS activating mutations and ARID1A and PTEN inactivating mutations[232,233]. A study conducted in 2023, with mice, was capable of successfully developed carcinoma by inducing the knockout (KO) of ARID1A and PTEN in the epithelium of endometriotic cysts, which were formed by the transplantation of small uterine pieces onto the peritoneum or ovarian surface, having a EAOC developed as early as 4 wk after the KO[232]. Helping to consolidate and bring up more data surrounding the intrinsic correlation between the dysfunctions caused by endometriosis and the onset of ovarian cancer.

IMPLICATIONS FOR CLINICAL MANAGEMENT

Immunotherapy in ovarian cancer and endometriosis

Unlike the traditional strategies of killing tumor cells, immunotherapy is a treatment approach that utilizes cells, viruses, peptides, small molecules, or antibodies to activate or modulate the immune system to attack cancer cells[234]. This treatment has brought

about a significant transformation in the approach of various solid tumors, including malignant melanoma, non-small-cell lung cancer, and renal cell carcinoma. It has become the leading choice for managing recurrent or metastatic solid tumors, surpassing conventional chemotherapy and targeted therapy[235].

Over the past few decades, immunotherapy has surfaced as a hopeful treatment alternative for gynecologic malignancies, including ovarian cancer. The first line of treatment for ovarian cancer is usually cytoreductive surgery combined with chemotherapy[236]. However, chemoresistance is one of the most prevalent factors for the poor prognosis of this pathology, especially when associated with metastatic capacity and clinical course of the disease[237]. As ovarian cancer is a tumor with an extremely immunosuppressive microenvironment, the immunological approach shows great promise[238]. Currently, immune strategies for the treatment of ovarian cancer are being tested in clinical trials, and include checkpoint inhibition, cancer vaccines, oncolytic virotherapy and adoptive cell therapy. Figure 3 depicts a simplified schematic illustrating of current possible immunotherapy approaches to ovarian cancer.

Immune Checkpoint Inhibitors

Effective immunotherapy for ovarian cancer hinges on activating antigen-presenting cells, reducing the immunosuppressive microenvironment, and enhancing the performance of effector T cells. The T cell–mediated immune response is controlled through both inhibitory and activating signals, and immune checkpoint receptors play a crucial role in restraining T cell activation to prevent excessive stimulation. Nonetheless, numerous types of tumors exhibit immune checkpoint expression, which results in immune evasion. Consequently, inhibitors targeting immune checkpoints play a significant role in immunotherapy[239]. Up until now, the most promising immune checkpoint inhibitors for solid tumors have been antibodies that hinder CTLA4, PD-1 and PD-L1, which are presented in some drugs approved by The Food and Drug Administration, such as CTLA4 antibodies (Ipilimumab), PD-1 antibodies (Pembrolizumab and Nivolumab), and PD-L1 antibodies (Avelumab, Atezolizumab and Durvalumab)[239,240]. However, the clinical application of checkpoint inhibitors in

ovarian cancer has yielded limited success, with single-agent response rates in clinical trials typically hovering around 6-15%[241-243]. As single agents, the results in ongoing clinical trials showed modest effects of ICIs in ovarian cancer, limiting its approval for use in patients with OC[244].

Another potential target of immune checkpoint blockade is B7-H3, which is an immunosuppressive molecule present on tumor cells but absent in host cells, and researchers have explored the therapeutic impacts of blocking B7-H3 and PD-1 in the context of cancer[238]. The results indicate that, in ID8 tumor-bearing mice with ovarian cancer, it is B7-H3 inhibition, not PD-1 blockade, that prolongs the median survival time[245].

Immune checkpoint inhibitors monotherapy has limited anti-tumor effects in ovarian cancer, and the efficacy of ICI depends on the condition of TILs and the expression of specific molecules. Hence, ideal candidates for this strategy should be well-chosen. To surpass these barriers, combining therapies are being tested to improve the anti-tumor activity in ovarian cancer[246].

PARP Inhibitors

The poly (ADP-ribose) polymerase (PARP) is a well-acknowledged detector of DNA damage, renowned for its involvement in repairing DNA base excision and single-strand breaks (SSB). PARP inhibitors have emerged as a novel targeted therapy for ovarian cancer, especially for women carrying BRCA1 and BRCA2 mutations or individuals lacking a functional homologous recombination repair pathway[247]. Cells with impaired homologous recombination are vulnerable to PARP inhibitors. BRCA1 and BRCA2 are tumor suppressor genes known for their essential involvement in DNA repair, as they create a complex responsible for homologous recombination repair[248]. The FDA has sanctioned various PARP inhibitors for use, and some of them are currently under investigation in clinical trials. These include olaparib, niraparib, rucaparib, veliparib, and talazoparib[249]. Despite the encouraging advantages offered by PARP inhibitors, numerous limitations persist, as shown in some studies[250-253]. Future research should prioritize exploring and developing combinations that can

amplify the impact of PARP inhibitors, such as antiangiogenic agents and ICIs combining therapies[254].

Adoptive Cell Therapy

Adoptive cell therapy (ACT) primarily relates to the utilization of chimeric antigen receptor (CAR)-modified T cells, T-cell receptor (TCR)-engineered T cells, natural tumor-infiltrating lymphocytes (TILs), CAR-NK cells, and CAR-macrophages. ACT has brought about a significant breakthrough in treating blood-related tumors. However, when it comes to solid tumors like ovarian cancer, ACT appears to be inadequate in inducing substantial anti-tumor responses[255]. Up until now, there has not been a notable therapeutic effectiveness. The primary challenges lie in the weak binding affinity and inconsistent presence of targetable surface antigens, as well as obstacles related to the infiltration and viability of CAR-T cells[256]. Therefore, additional clinical data is necessary to verify their effectiveness in individuals with ovarian cancer.

Cancer vaccine

Vaccination plays a crucial role in immunotherapy, offering advantages to individuals affected by diverse forms of cancer, and there is extensive research into therapeutic vaccines in the context of ovarian cancer. The investigation into the potential of single application of a cancer vaccine for ovarian cancer is ongoing, and this includes the examination of various types of vaccines such as peptide vaccines, whole tumor cell vaccines, cancer stem cell vaccines (CSCs), antigen-presenting cell (APC) vaccines, DNA/RNA vaccines, bacterial vaccines, and more[255]. Many of these vaccines enhance the body's immune response against ovarian cancer, but clinical evidence has only demonstrated modest effectiveness in the majority of patients[257-259]. It is feasible to assess the therapeutic potential in a broader group of patients. The use of dendritic cell-based vaccines is another treatment approach, which is also under investigation in patients with ovarian cancer, being shown that it can produce improved outcomes[236,246]. However, the evaluation of the clinical use of vaccines in cancer patients has certain limitations, such as the heterogeneity of antigen expression within a tumor[260,261].

Immunotherapy in endometriosis

The immune system has a significant role in endometriosis. Therefore, immune therapy holds promise as a potential treatment approach for this condition. The reduced macrophagic phagocytosis observed in the serum and peritoneal fluid of individuals with endometriosis is a significant contributor to the disruption of immune balance[262]. In this sense, the expression of CD47 is used by macrophages to distinguish "self" or "non-self" cells. The CD47 site inhibitor disrupts this signal, enabling macrophages to carry out regular phagocytosis[263]. Clinical observations have revealed a substantial elevation in CD47 Levels within the ectopic endometrial tissue of individuals with endometriosis[264]. Immunotherapy targeting the CD47-SIRPa signaling pathway appears to show promise in the management of endometriosis[265].

Furthermore, it has been reported that exosomes originating from endometriosis can induce a shift in macrophage phenotype toward M2 polarization, leading to a reduction in macrophage phagocytic activity both in laboratory settings (in vitro) and within the body (in vivo)[262]. As a result, employing anti-exocrine therapy for individuals with endometriosis appears to have a notable effect on attracting macrophages to ectopic lesions[266]. This therapy can decrease the presence of M2-type macrophages, leading to an overall enhancement in macrophage-mediated phagocytosis of ectopic endometrial cells[267].

Moreover, encouraging NK cell cytotoxic activity is a potential novel therapeutic approach for managing endometriosis[268]. The treatment is based on NK inhibitory receptors that dampen their response to ectopic or malignant cells. The PD1, one such receptor that interacts with the PDL1 Ligand has already shown promise in cancer immunotherapy[269]. This approach aims to potentially enhance the rescue of endometrial cells, counteract the suppression of NK cells' regulatory function, and enable the elimination of misplaced endometrial cells. The proposed immunotherapy strategy suggests utilizing existing medications, commonly employed for conditions like cancer, to aid in identifying and prompting the removal of endometrial cells through apoptosis[270].

Besides this, vitamin D has been noted for its immunomodulatory properties in the medical management of endometriosis [39]. In a recent animal model study focused on endometriosis, it was observed that the use of synthetic vitamin D derivatives significantly curbed the progression of both endometriosis and peritonitis[271]. Considering the role of inflammatory cytokines in the development of endometriosis, recent studies have suggested that the impact of 1,25(OH)2D3 on the cytokine production within human endometrial stromal cells could be a contributing factor to the therapeutic benefits of this compound in treating endometriosis. Additionally, another investigation has shown that 1,25(OH)2D suppresses the immune response of Th1 cells while promoting the response of Th2 cells. It achieves this by inhibiting the release of IL-12, IL-2, and TNF from T cells, macrophages, and DCs, respectively[272].

Targeting macrophages and inflammatory mediators

The direct involvement of tumor-associated macrophages (TAMs) in the oncogenesis and prognosis of ovarian cancer based on the imbalance in polarization between M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages has been previously established[273]. Thus, it was discovered that the presence of a majority of M2 (anti-inflammatory) macrophages in the ovarian environment is a determining factor for a worse cancer prognosis, through the interaction of specific receptors of these cells with the immune system and also through the stimulation of various cytokines, generating an environment more conducive to the establishment of ovarian cancer of all types, including endometriosis-associated ovarian carcinoma (EAOC)[274]. In this way, investigation of new ways of interfering in this process using the specific receptors expressed on M2 macrophages and the cytokines produced can be a new approach in order to achieve better therapeutic results and greater survival[275].

CD47- SIRPα

CD47 is a glycoprotein that is very present in the tumor environment and exerts its inhibitory activity by binding to its counter-receptor, the signal regulatory protein-α (SIRPα), expressed in macrophages[276,276]. Liu R *et al.* conclude that the impact of this inhibitory process is a reduction in phagocytosis by these macrophages (known as the

"don't eat me signal"), which culminates in the progression of the tumor microenvironment, thus serving as one of the strategies for inhibiting immune activity by tumor cells[277].

Based on this principle, therapies based on blocking CD47 (anti-CD47) and SIRPα have emerged, which aim to interfere with the inhibitory process[278]. Son *et al.* demonstrated that these two therapies are promising in the treatment of solid cancers, such as ovarian cancer, and have shown good progress in terms of improved anti-tumor activity of macrophages based on the established blockade of inhibitory receptors[278].

Kaur *et al.* reported a series of preclinical studies in mice and humanized clinical studies of anti-CD47 therapy, bringing a positive result, since both the preclinical study and the humanized clinical studies showed promising data in relation to limiting tumor cell growth[279].

Sikic *et al.* also demonstrated a promising result when they carried out a phase I study in humans using the anti-CD47 antibody Hu5F9-G4, which showed safety, tolerability and also tumor regression in some patients undergoing therapy[280]. It is also important to note that Tian *et al.* were able to demonstrate that anti-CD47 therapy has a promising future in improving the activity of innate immunity and the oncolytic process[281].

In addition, Li *et al.* also reported a study showing high CD47 expression in patients with endometriosis, in which CD47 blocking treatment resulted in an increase in the phagocytic process by macrophages and also in an increase in apoptosis of endometrial stromal cells, representing a protective effect against the development of endometriosis-associated ovarian cancer (EAOC)[264]. Figure 4 depicts a simplified schematic illustrating of CD47- SIRP-based immunotherapy.

CSF-1/CSF-1R

The colony-stimulating factor-1 receptor (CSF-1R) is a receptor that exists in several human cells during homeostasis, but is overexpressed in tumor-associated macrophages in several types of cancer, including ovarian cancer[282]. The specific ligand of this glycoprotein is CSF-1, which is found in high levels in tumor cells and the binding of these two receptors culminates in an oncogenic role through the release of

growth factors and substances that stimulate the cellular differentiation of macrophages into M2[282]. In this way, the relationship between both glycoproteins has become the object of study for the development of new therapeutic techniques, based on the inhibition of these receptors, with the aim of reducing TAMs and tumor growth[283].

Based on this premise, Ries *et al.* used a monoclonal antibody directed at blocking CSF-1R in cancer patients, with the aim of manipulating the activity of TAMs, and the result was promising, since a reduction in tumor-associated macrophages was achieved in patients as well as an increase in the levels of TCD8/CD4 cells in animal models[284].

Lu *et al.* conducted a study in murine models of ovarian cancer and associated a CSF-1R inhibitor with docetaxel and concluded that treatment with the inhibitor alone resulted in increased cell apoptosis of tumor-associated macrophages due to the repolarization effect, transforming them into M1[285]. Combined treatment resulted in a significant reduction in tumor growth, a reduction in TAMs and increased levels of TCD8+ cells[285].

Finally, Xiaocui *et al.* identified a significant presence of the glycoprotein CSF-1 in patients with endometriosis, which resulted in an elevated appearance of macrophages with an anti-inflammatory effect and, consequently, a depletion of the activity of the immune system in this environment, leaving it vulnerable to the appearance of possible ovarian cancer[286]. This study also found that the use of an anti-CSF-1 antibody reduced these negative effects, demonstrating the promising activity of this immunotherapeutic field[286]. Figure 5 depicts a simplified schematic illustrating of CSF-1/CSF-1R-based immunotherapy.

CCL2/CCR2

The C-C motif chemokine ligand 2 (CCL2)/C-C motif chemokine receptor 2 (CCR2) axis is involved in the recruitment of monocytes from the bloodstream to the tumor environment, since CCL2 is a chemokine that attracts CCR2+ monocytes, which will become M2 macrophages when they arrive in the tumor environment[287].

This axis has also become a therapeutic target, as demonstrated by Miyamoto *et al.* who based their study on blocking the CCL2/CCR2 interaction[288]. The study achieved a

positive result in mice by reducing M2 macrophages and increasing TCD8+ cells and IFNγ by inhibiting the CCL2/CCR2 relationship[288].

In addition, it is also necessary to highlight the relationship between CCL2 and endometriosis, as reported by Hogg *et al.*, who reported that high levels of CCL2 are present in endometriosis, thus resulting in greater recruitment of macrophages that may impact on the inflammatory environment and contribute to the process of malignization of the lesion, generating ovarian cancer[289].

CXCR4-CXCL12

C-X-C receptor 4 (CXCR4) is a chemokine that functions through its binding to the C-X-C motif ligand 12 (CXCL12), derived from stromal cells, and the activation of this axis is closely linked to the initiation and progression of ovarian tumors from the invasion of ovarian cancer cells[290,291].

With this in mind, Xue J *et al.* used an antagonist of the CXCR4 receptor in order to reduce the impacts derived from its activity and achieved promising results, which are the blocking of the activation of the NF-κB signaling pathway and, consequently, a reduction in tumor growth and a reduction in the possibility of metastasis[292].

Pharmacological modulation of TAMs

The polarization of macrophages and their influence on the oncogenesis and progression of ovarian cancer has also extended the discussion into the field of pharmacological modulation, with the clear aim of finding a way to revert M2 macrophages into M1, promoting anti-tumour activity and, consequently, regression of the already established tumour[293,294].

In their study, Bolli E *et al.* used imidazoquinoline (IMDQ), an agonist of the Toll 7/8 receptor, which was coupled to an antibody and infused intravenously into patients with ovarian cancer with the aim of blocking the macrophage mannose receptor (MMR) and causing a repolarization of macrophages from M2 to M1[24]. The results of the study were promising and showed a reduction in tumor growth, associated with an increase in the pro-inflammatory process derived from the change in the types of macrophages employed in the environment[295].

On the other hand, the study by Xiao *et al.* addressed the development of a nanodrug specific for tumor-associated M2 macrophages, from siRNA IKKβ (an activator of NF-kβ and STAT6, participants in the polarization process), with the clear objective of inhibiting STAT6, thus resulting in a safe conversion of macrophages and the reduction of tumor growth with the activation of the antitumor axis in an *in vivo* experiment[296].

Finally, Hsieh *et al.* presented a study based on the use of vorinostat in mice with EAOC, in which the result was a reduction in the tumor from the inhibition of the polarization of M2 macrophages[297]

Anti-inflammatory agents in the context of endometriosis and ovarian cancer.

Cyclooxygenase-2 (COX-2) is an enzyme directly linked to inflammatory processes from arachidonic acid and the consequent production of prostaglandins[298]. COX-2 has been described as an overexpressed marker in ovarian cancer, playing a direct role in the poor prognosis of patients with this type of cancer, since it participates in the migration and invasion of tumor cells[299]. In addition, Lai ZZ *et al.* bring up the direct involvement of COX-2 in endometriosis, since, as found in the study, cyclooxygenase-2 promotes increased cell proliferation, reduced apoptosis and increased angiogenesis, factors that are also linked to oncogenesis[300,301].

Thus, studies such as that by Li W *et al.* show the importance of investigating the impact of anti-inflammatory drugs on ovarian cancer, since, as reported in this analysis, a positive effect was found when associating a COX-1 inhibitor with Cisplatin or Taxol in order to reduce angiogenesis in ovarian cancer in murine models[302].

Therefore, the direct involvement of COX-1 and COX-2 in the oncogenesis and progression of ovarian cancer, as well as in the progression of endometriosis, has already been established. Therefore, new clinical studies with a large number of patients using COX inhibitors are still needed in order to establish an even more efficient treatment for ovarian cancer and endometriosis.

CONCLUSION

Endometriosis and ovarian cancer are still underreported diseases. This means that much involved in their pathophysiology and correlations still remains hazy. This is also due to the lack of feasible and efficient diagnostic resources in clinical practice. Especially with regard to the applicability of biomolecular resources to support the diagnosis and monitoring of those pathologies.

Future studies should aim to better elucidate the roles of oxidative stress, inflammation, and estrogen in EAOC development. Existing evidence suggests a shared microenvironment between endometriosis and EAOC in terms of cytokines and mediators, but further research is necessary to confirm a direct link between the two. Additionally, further research should focus on discovering biomarkers capable of identifying endometriosis cases with oncogenic potential, with the aim of detecting premalignant lesions, and consequently develop better interventions in order to decrease the incidence of EAOCs.

Research efforts should prioritize establishing model systems for endometriosis. These investigations could yield valuable knowledge about risk factors, molecular traits specific to subtypes, novel therapeutic testing, and the factors that contribute to the development of endometriosis-associated ovarian cancers (EAOCs). Additionally, it is crucial to emphasize the necessity of current interventions that lower the risk of ovarian cancer, including endometrioid carcinoma and clear cell carcinoma.

Moreover, non-invasive methods to help diagnose the disease should be a priority, as well as clarification of the genetics and genomics that control disease development, environmental contributions, and the involvement of the immune system. Other than that, well-designed clinical trials are essential to determine which therapies are safe and effective, and which markers and targets on the immune system may be useful in treatment and management. And confirmation of the veracity of these biomarkers can help in the development of true research, with larger populations, to understand endometriosis in particular.

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