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Clinicopathological characteristics, molecular features and novel diagnostic strategies for the detection of malignant transformation of endometriosis (Review)

HIROSHI KOBAYASHI[1,2]

[1]Department of Gynecology, Ms.Clinic MayOne, Kashihara, Nara 634-0813; [2]Department of Obstetrics and Gynecology, Nara Medical University, Kashihara, Nara 634-8522, Japan

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Abstract. Endometriosis is a benign gynecological disease that affects women of reproductive age. Although malignant transformation of endometriosis is rare, physicians must be aware of this due to the high incidence of clear cell carcinoma of the ovary (CCC) in Japan. The most prevalent histological subtype of ovarian cancer is CCC (~70%) followed by endometrioid carcinoma (30%). The present review discusses the clinicopathological and molecular features of endometriosis-associated ovarian cancer (EAOC) as well as prospects for novel diagnostic strategies. Papers published between 2000 and 2022 in the PubMed and Google Scholar databases were included. Contents of the endometriotic cyst fluid may be involved in carcinogenesis, although the underlying mechanisms are largely unknown. Some studies have proposed a possible mechanism wherein excessive hemoglobin, heme and iron could cause an imbalance in intracellular redox homeostasis in endometriotic cells. Combined with DNA damage and mutations, the imbalances may induce the development of EAOC. Endometriotic cells evolve to adapt to the prolonged unfavorable oxidative microenvironmental stress. On the other hand, macrophages enhance the antioxidative defense mechanism and protect endometriotic cells against oxidative damage through intercellular crosstalk and signaling pathways. Therefore, changes in redox signaling, energy metabolism and the tumor immune microenvironment could be the key elements in the malignant transformation of certain endometriotic cell clones. Additionally, non-invasive bioimaging (i.e., magnetic resonance relaxometry) and biomarkers (i.e., tissue factor pathway inhibitor 2) may be promising tools for early-stage detection of the disease. In conclusion, the present review summarizes the latest advancements in research on the biological characteristics and early diagnosis of malignant transformation of endometriosis.

Correspondence to: Dr Hiroshi Kobayashi, Department of Obstetrics and Gynecology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan
E-mail: hirokoba@naramed-u.ac.jp

Key words: diagnosis, endometriosis, malignant transformation, metabolism, oxidative stress

Contents

1. Introduction

Endometriosis is a common gynecological disease in women of reproductive age that is characterized by the development of functional endometrial tissue located outside the uterus (1). Endometriosis is a pathologically benign disease that is classified into ovarian endometriosis, superficial peritoneal disease, or deep infiltrating endometriosis. Endometriosis most commonly affects the ovaries and is associated with pelvic pain, infertility, or malignant transformation (1). Endometriosis is a possible precursor lesion of endometriosis-associated ovarian cancer (EAOC), including clear cell carcinoma of the ovary (CCC) and endometrioid ovarian carcinomas (2). CCC is the most common subtype of EAOC in Japan (70%) followed by endometrioid carcinoma (3).

The molecular mechanisms underlying the malignant transformation of endometriosis remain unclear; however, redox homeostasis imbalance might be involved. Endometriotic cysts contain high levels of iron, and endometriotic cells survive in reactive oxygen species (ROS)-rich environments (4). Hemoglobin, heme, and iron derivatives cause DNA damage and mutations, which promotes endometrial cell survival and proliferation and causes ectopic implantation (5-7). However, high levels of iron lead to cell death due to severe oxidative stress. It is necessary to elucidate the adaptive mechanisms that allow endometriotic cells to survive harsh environments and

the key mediators that regulate redox homeostatic balance (7). Due to the greater number of patients with CCC in Japan compared with Western countries (25% vs. 8%), special attention has been paid to ensure the early diagnosis of malignant transformation of endometriosis (3).

The aim of this review is to summarize information on the clinicopathological characteristics, molecular mechanisms underlying redox homeostasis, and innovative diagnostic methods for malignant transformation of endometriosis. Finally, we discuss the current challenges and future directions.

2. Literature search

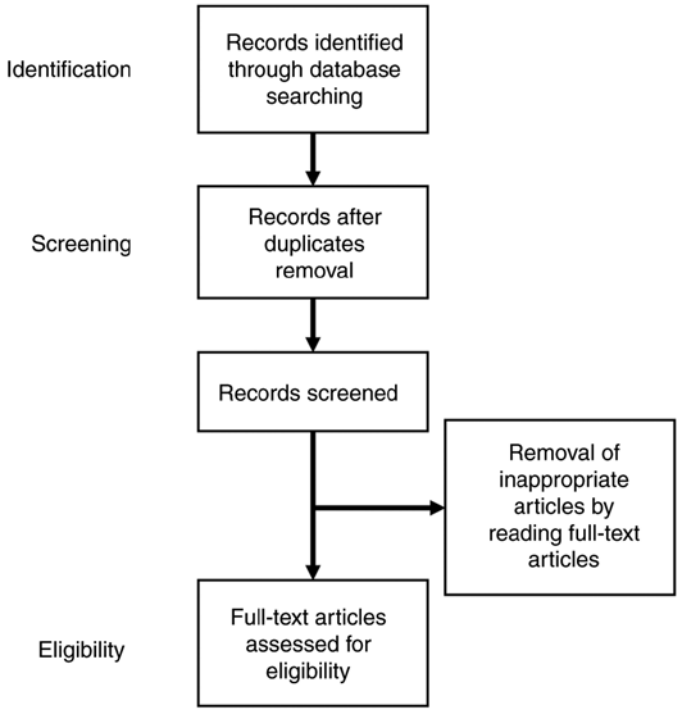
Search strategy and selection criteria. A computerized literature search was performed to identify relevant studies in English. The PubMed and Google Scholar electronic databases were searched for studies published between January 2000 and February 2022. The search terms included *endometriosis*, *endometriosis-associated ovarian cancer*, *oxidative stress*, *antioxidant*, *energy metabolism*, *macrophages*, *imaging*, and *serodiagnosis*. The references of each article were searched to identify potentially relevant studies. Publications of original studies and review papers were included. Given the heterogeneity in the research theme, data from studies were synthesized using a descriptive review design with narrative methods. Fig. 1 shows the first identification phase that includes records identified through a database search. Terms in the titles and abstracts were searched during the first screening. During the second screening, duplicates were removed, and titles, abstracts, and full-text articles were read to remove inappropriate papers. The final eligibility phase included full-text articles for analysis after excluding those wherein detailed data cannot be extracted.

3. Current understanding of malignant transformation of endometriosis

Current challenges in the malignant transformation of endometriosis in clinical practice. Most cases of endometriosis are benign and regress spontaneously after menopause. Sampson reported the first case of a malignant neoplasm arising from pre-existing endometriosis in 1925 (8). Malignant transformation of endometriosis is rare, with an incidence of approximately <1.0% (2,9). Advances in genomics and molecular biology have made it possible to elucidate the potential mechanisms in the malignant transformation of endometriosis. Some researchers have proposed the clonal evolution model, wherein normal endometrial cells transform into neoplastic cells via endometriosis and atypical precursors based on oncogenic driver gene mutations (i.e., AT-rich interacting domain-containing protein 1A [ARID1A] gene) (10). Murakami *et al* (11) recently proposed a carcinogenic mechanism that 'EAOC might not occur as a result of malignant transformation of endometrial cysts and might be caused by eutopic endometrial glandular epithelial cells that are refluxed to engraft in the ovary'. Genomic and epigenomic studies are required to better understand why and how endometriosis causes malignant transformation.

Epidemiological studies revealed that hysterectomy and unilateral oophorectomy reduces the risk of EAOC by 50-80% (12,13). Therefore, more than half of cases of EAOC originate from endometrial tissue fragments that have been regurgitated during menstruation, while other cases may have been caused by already existing endometriotic cysts (11). In fact, histopathological examination revealed that some cases of EAOC showed a continuous transition from benign endometriosis to atypical endometriosis and finally to invasive carcinoma (14-16). The prevalence of atypical endometriosis varies considerably, ranging from 1.7 to 40% (17-20). The difference in the results may be due to the lack of a unified international consensus regarding the definition of atypical endometriosis. In clinical practice, endometriosis is diagnosed based on clinical symptoms combined with diagnostic techniques, including transvaginal ultrasound (TVS) and blood biomarkers (e.g., carbohydrate antigen 125 [CA-125]) (21). TVS is the first-line diagnostic method for discriminating benign from malignant ovarian tumors. As shown in Fig. 2, EAOC is more common in women aged over 40 years, and the size of the cyst is larger than that of endometriosis (9). Benign ovarian endometriosis may pertain to any of the following: 1) pathologically benign endometriosis; 2) endometriosis with atypical lesions; and 3) endometriosis with clinically undetectable early-stage ovarian cancer. Although endometriosis with atypical lesions and with undetectable early-stage cancer may develop into ovarian cancer, patients with these conditions cannot be diagnosed as having cancer. Therefore, current screening options for early detection of EAOC are warranted.

Ovarian cancer initiation and progression via persistent oxidative stress mediated by iron overload. Oxidative stress is caused by an imbalance in the redox homeostasis between ROS overproduction and the antioxidant defense system (22). Iron-induced redox imbalance plays an important role in the pathophysiology of endometriosis, resulting in either cell death (anti-tumorigenic) or survival (pro-tumorigenic) (4,6,7). Repeated hemorrhage occurs in endometriotic cysts and in the peritoneal cavity during menstruation (2,7). Hemoglobin, heme, and iron are released following hemolysis from red blood cells (2,23). Cellular iron is imported through enhanced divalent metal transporter-1 (DMT1) and exported through ferroportin (FPN) (24). The differential expression of transporters (i.e., DMT1 upregulation and FPN downregulation) is associated with increased levels of intracellular iron (24). Iron content is reportedly elevated in the peritoneal cavity of women with endometriosis, in endometriotic cysts, in macrophages throughout the stroma of endometriotic cysts, and in the fimbriae of the fallopian tubes (24,25). Autoxidation (22) and the Fenton reaction (26) from the transformation of ferrous Fe^{2+} (oxyhemoglobin) to ferric Fe^{3+} (methemoglobin) produce large amounts of superoxide radicals (O_2^-) and hydroxyl radicals (OH). Accumulation of oxidative stress biomarkers (e.g., methemoglobin, 8-hydroxy-2'-deoxyguanosine, and lipid ROS) and inactivation of antioxidant enzymes (e.g., glutathione peroxidase 4) have been noted in endometriotic cysts (22,27). High levels of hemoglobin, heme, and iron derivatives in endometriotic cysts induce redox imbalance (22), leading to cell death through persistent DNA damage (7). Moreover, excessive accumulation of lipid ROS triggers ferroptotic cell death in an iron-dependent manner (28). In contrast, antioxidants protect cells from DNA damage by neutralizing



Key words	1+3+4+5	1+3+4+6	1+3+7
Identification	145	20	11
Records after duplicates removal	20	0	1
Records screened	125	20	10
Removal of inappropriate articles	90	11	3
Eligibility	35	9	7

Key words	2+3+4+5	2+3+4+6	2+3+7	2+8
Identification	49	3	17	78
Records after duplicates removal	5	0	1	5
Records screened	44	3	16	73
Removal of inappropriate articles	8	1	6	42
Eligibility	36	2	10	31

Figure 1. Number of articles identified. This figure shows the number of articles identified by key word combinations and the number of records identified through database searching, records after duplicate removal, records screened, removal of inappropriate articles by reading full-text articles and full-text articles assessed for eligibility. Key words: 1, 'endometriosis'; 2, 'endometriosis-associated ovarian cancer'; 3, 'oxidative stress'; 4, 'antioxidant'; 5, 'energy metabolism'; 6, 'macrophages'; 7, 'imaging'; and 8, 'serodiagnosis'. For example, '1+3+4+5' in key words means a combination of the key words 1 ('endometriosis'), 3 ('oxidative stress'), 4 ('antioxidant') and 5 ('energy metabolism'). As a result of literature search using '1+3+4+5', 145 articles were included in the identification step, 125 articles were selected in the screening step and finally 35 articles remained in the eligibility step.

excess ROS (29). A sublethal dose of iron overload participates in mutagenesis and aberrant pro-tumorigenic signaling to activate cell survival and promote carcinogenesis (29,30). Thus, low to moderate oxidative stress may contribute to the development of EAOC (26,27,29-31). This suggests that not only endometriosis but also conditions of iron overload, such as hemochromatosis, viral hepatitis B and C, and asbestos exposure, are risk factors for cancer development (32).

Metallobiology revealed diverse features involved in redox imbalance and the pathogenesis of malignant transformation of endometriosis (27). Compared to endometriosis, the contents of EAOC cysts have high antioxidant capacity due to lower levels of iron (14.2±36.6 mg/l vs. 244.4±204.9 mg/l) and higher levels of oxyhemoglobin (33) (Fig. 2). The contents of endometriotic and EAOC cysts are often dark and light brown, respectively, which may reflect iron concentrations and hemoglobin species. A study examining the expression of iron transport proteins showed that iron excretion is greater in CCC cells than in endometriotic cells (24), indicating that the intracellular levels of iron in CCC cells are maintained at low levels. Iwabuchi *et al* (27) provided a detailed characterization of hemoglobin species, such as methemoglobin and oxyhemoglobin, in endometriotic and EAOC cysts. The major components of hemoglobin species in endometriotic and EAOC cysts are methemoglobin and oxyhemoglobin, respectively (27) (Fig. 2). High levels of antioxidants allow EAOC cells to escape the lethal effects of excessive oxidative stress-induced cell death. Thus, iron levels should be maintained within a narrow range to prevent endometriotic cell death, which may in turn increase the risk of cancer progression when the cellular antioxidant capacity exceeds ROS production (2,6,27,29).

Dual role of antioxidants in the different stages of malignant transformation of endometriosis. Nagayasu *et al* (34) presented a comprehensive review on the endogenous antioxidant

	Endometriosis	EAOC
Transvaginal ultrasound	TVS shows ground-glass appearance of the cyst fluid.	TVS shows papillary projections with a low-level echogenicity of the cyst fluid.
Cyst fluids	A dark brown appearance	A light brown appearance
Age	<40 years	40-60 years
Size	Small-moderate	Moderate-large
Iron	244.4 ± 204.9 mg/l	14.2 ± 36.6 mg/l
Hemoglobin	Methemoglobin > oxyhemoglobin	Methemoglobin < oxyhemoglobin
Redox balance	Oxidative stress > antioxidants	Oxidative stress < antioxidants
MR relaxometry R2 value	>12.1 /sec	<12.1 /sec
CA125	<100 U/ml	35-100s U/ml
TFPI2	<191 pg/ml	>191 pg/ml (CCC, >270 pg/ml)

Figure 2. Clinicopathological, biological and biochemical characteristics of patients with endometriosis and clear cell carcinoma. CCC, clear cell carcinoma of the ovary EAOC, endometriosis-associated ovarian cancer; MR, magnetic resonance; R2, relaxometry value 2; TFPI2, tissue factor pathway inhibitor 2; TVS, transvaginal ultrasound.

defense systems in endometriosis. The generated ROS can be scavenged by enzymatic (e.g., superoxide dismutase [SOD], catalase, reduced glutathione [GSH], glutathione peroxidase [GPX], and thioredoxin) and non-enzymatic (e.g., estradiol, melatonin, vitamin E, and vitamin C) antioxidants (35,36). Increased levels of oxidants (e.g., iron, ROS, nitric oxide [NO], lipid peroxidation, or advanced oxidation protein products) and decreased levels of antioxidants (e.g., SOD, catalase, GSH, GPX, glutathione reductase, total antioxidant capacity, vitamin C, or vitamin E) were observed in the serum, peritoneal fluid, follicular fluid, and tissue samples collected from patients with endometriosis compared to those in patients without endometriosis (37-39). Estradiol is a potent antioxidant that overcomes excessive oxidative stress by directly scavenging ROS or increasing the expression of SOD (34), and it is also involved in the development, maintenance, and progression of endometriosis. This may be the reason why endometriosis spontaneously regresses after menopause.

Overproduction of ROS and upregulation of endogenous antioxidant genes and signaling pathways are the main biological features of human cancers (29). Antioxidants have a dual role in tumor development and progression as both a tumor suppressor and promoter (29). Since nuclear factor erythroid 2-related factor 2 (NRF2) and CD44 variant isoform 9 (CD44v9) are well-studied regulators of antioxidants, we explain the role of these genes as an example. In genetically engineered mouse model, antioxidant (e.g., NRF2) depletion promotes carcinogenesis, demonstrating that antioxidants are involved in inhibiting cancer initiation and development (40). For example, vitamin supplementation may decrease the risk of developing human cancers (41). In contrast, treatment with antioxidant inhibitors reduced the growth of pre-existing cancer in xenograft animal models (42). Antioxidant inhibitors increase intracellular ROS and accelerate oxidative damage that induces cell death. For example, redox homeostasis in ovarian cancer is regulated by endogenous antioxidants, including NRF2 and CD44v9 (29). Concurrent inhibition of CD44v9 and NRF2 triggers apoptotic cell death via the ROS-dependent p38 and p21 pathways (29). Animal experiments revealed that sublethal oxidative stress (low to moderate toxicity) is involved in the early phase of cancer initiation, while sufficient antioxidant capacity is necessary in the late phase of cancer progression. Similar results have been observed in changes in redox homeostasis in the process of malignant transformation of endometriosis (22,43). In the EAOC group, 8-OHdG levels in cystic fluids were significantly decreased, while levels of antioxidants (e.g., heme oxygenase-1 [HO-1], cytochrome P450 family, and glutathione transferase family) were increased compared with those in the endometriosis group (22,43). EAOC cells might be protected against excessive oxidative stress by an effective antioxidant defense (22). A fine-tuned pattern in redox homeostasis supports the idea that pro- and antioxidants may be involved in the initiation and progression of EAOC, respectively (7,23). EAOC is a disease that involves redox homeostasis and has predominant antioxidant capacity. Taken together, iron homeostatic pathways contribute to cancer initiation and progression via two major processes (Fig. 3). In the first step, hemoglobin, heme, and iron cause DNA damage and mutations by producing ROS, leading to cancer initiation. In the next step, antioxidants influence cancer progression.

Altered survival signals in endometriosis and EAOC. Energy production is required for cell adaptation and survival in stressful environmental conditions. Glucose is metabolized through glycolysis and oxidative phosphorylation (OXPHOS) to produce adenosine triphosphate (ATP), an important energy source. Endometriosis and EAOC, especially CCC, are characterized by common metabolic and molecular alterations (5,44,45). The key metabolic features include increased glucose uptake, anaerobic glycolysis, lactate production, and metabolic conversion from mitochondrial OXPHOS to aerobic glycolysis (44-47). Kirsten rat sarcoma (KRAS) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit

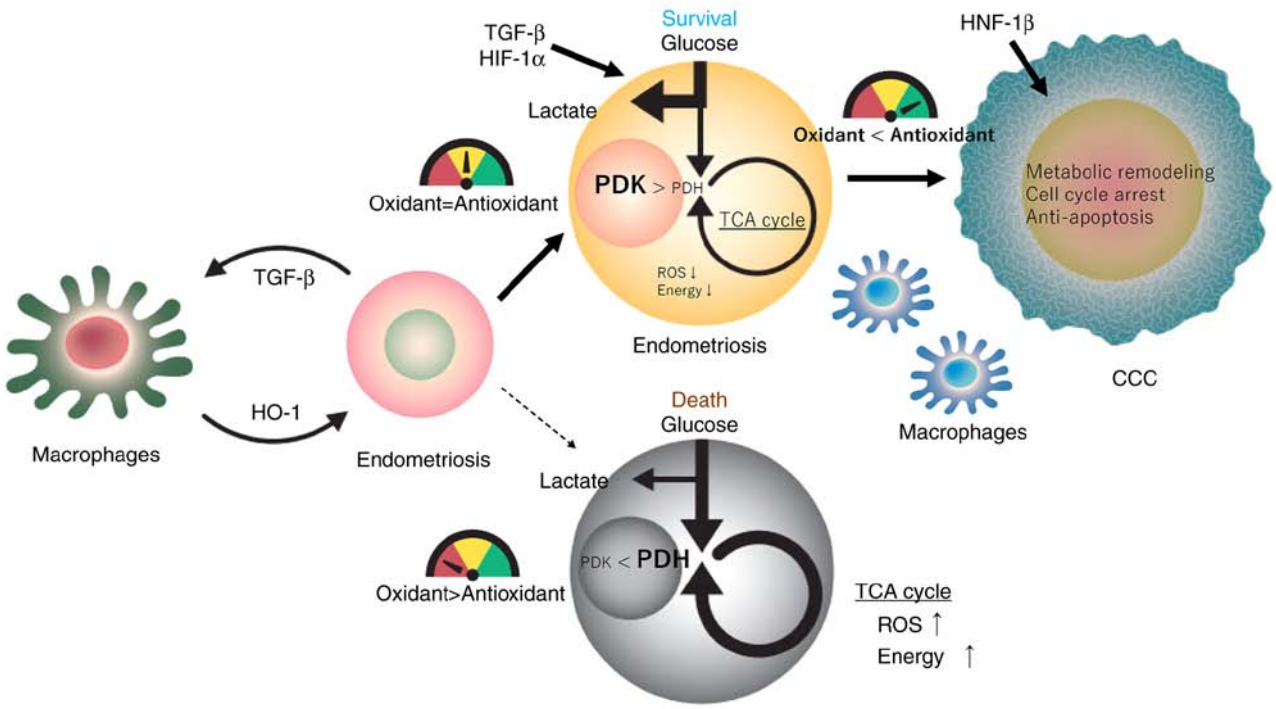


Figure 3. Redox balance and metabolic switch regulating endometriotic cell fate (survival or death) during malignant transformation. Redox and metabolic changes caused by exposure of endometriotic cells to hemoglobin, heme and iron-induced oxidative stress are described. Excessive oxidative stress and a metabolic shift to mitochondrial oxidative phosphorylation interfere with biological processes associated with endometriotic cell growth and survival (oxidant > antioxidant). Macrophages protect endometriotic cells from oxidative injury through the upregulation of HO-1. TGF-β and HIF-1α induce glycolysis in endometriotic cells and are associated with a metabolic shift to anaerobic glycolysis, reduction of ROS levels, and increased survival (oxidant=antioxidant). HNF-1β prevents oxidative damage via metabolic remodeling, cell cycle arrest and anti-apoptosis (oxidant < antioxidant). Redox imbalance may be directly or indirectly involved in the malignant transformation of endometriosis. The redox meter shows red when oxidative stress is predominant, green when antioxidant is predominant and yellow when redox homeostasis is maintained. CCC, clear cell carcinoma of the ovary; HIF-1α, hypoxia-inducible factor-1α; HNF-1β, hepatocyte nuclear factor-1β; HO-1, heme oxygenase-1; PDK, pyruvate dehydrogenase kinase; PDH, pyruvate dehydrogenase; ROS, reactive oxygen species; TCA, tricarboxylic acid.

alpha (PIK3CA) mutations found in endometriosis and EAOC are associated with glucose metabolism (10,48,49). However, much of our understanding about energy metabolism by mutated genes has come primarily from *in vitro* studies using other types of cancer cells rather than those in endometriosis or EAOC.

Transforming growth factor-beta (TGF-β) and hypoxia-inducible factor-1alpha (HIF-1α) have attracted attention as potential target genes related to energy metabolism in endometriosis (44,45) (Fig. 3). TGF-β is expressed in response to hypoxia, mitochondrial stress, oxidative stress, or tissue damage (44,45,50). TGF-β signaling enhances aerobic glycolysis by promoting glucose transporter 1 (GLUT1) expression, which converts glucose to lactate (44,45,50). HIF-1α induced by TGF-β1 modifies the expression of metabolism-related enzymes (44). Overexpression of HIF-1 inhibits pyruvate dehydrogenase (PDH) activation via upregulation of pyruvate dehydrogenase kinase (PDK) expression (44,45). PDH converts pyruvate to acetyl-CoA and stimulates the transfer of acetyl-CoA to the tricarboxylic acid cycle, enhancing the formation of ATP. Therefore, TGF-β and HIF-1α are major players in the metabolic switch from OXPHOS to glycolysis through the PDK-PDH pathway (44,45). Mitochondria not only produce ATP but also induce oxidative stress via the overproduction of ROS. Therefore, cells that survive in unfavorable environments (e.g., endometriotic and cancer cells) suppress ROS production in favor of glycolysis over mitochondrial OXPHOS even if they reduce energy production (44,45). Indeed, endometriotic lesions had higher expression of TGF-β and HIF-1α genes compared with eutopic endometrium (44). TGF-β plays multiple roles in tumorigenesis, possibly through the activation of the HIF-1α-PDK-PDH pathway (44,45). A recent study demonstrated that overexpression of PDK2 was linked to poor prognosis in patients with CCC due to decreased production of mitochondrial ROS (51). Emerging evidence indicates that TGF-β, HIF-1α, and glycolysis-related genes play critical roles in the pathogenesis and progression of endometriosis and CCC.

Hepatocyte nuclear factor-1 beta (HNF-1β) is a transcription factor that is overexpressed in almost all CCCs (52). HNF-1β is expressed only in CCC and not in other types of ovarian cancers (53). HNF-1β is also expressed in approximately 40% of endometriotic lesions (52). HNF-1β is a promising new marker for molecular diagnosis of both benign and malignant lesions (54). Single-nucleotide polymorphisms (rs11651755) in HNF-1β also correlated with the risk for ovarian cancer and endometriosis (55,56). Several papers suggested that HNF-1β affects a wide range of biological events, including tumorigenesis. For example, HNF-1β promotes glycogen accumulation in the cytoplasm of normal and gestational endometrial and CCC cells (28,57). HNF-1β is a key regulator of gene expression regulating metabolic remodeling, cell cycle arrest, and anti-apoptosis. First, HNF-1β induces glucose uptake into CCC cells via the overexpression

of glucose transporter GLUT1 and facilitates glycolytic activity, lactate production, and glutathione synthesis, all of which are involved in the maintenance of redox homeostasis through enhanced anaerobic metabolism, decreased mitochondrial OXPHOS, and reduced ROS accumulation (46,53). Second, HNF-1β inhibits cell proliferation by blocking G1/S cell cycle progression through direct suppression of SMAD6-induced cyclin D1 expression (46,58). Furthermore, HNF-1β promotes G2/M cell cycle arrest in CCC cells through activation of the deubiquitinase ubiquitin-specific protease 28 (USP28)-Claspin-checkpoint kinase 1 (Chk1) signal transduction cascade (59). Finally, HNF-1β promotes cell proliferation and survival of endometriotic and CCC cells by upregulating the transcriptional regulator nuclear factor kappa B-induced antiapoptotic BCL2 gene expression (60,61). Thus, HNF-1β can facilitate DNA damage repair by inducing transient cell cycle arrest. HNF-1β protects endometriotic and CCC cells from oxidative damage, thereby contributing to the promotion of cell growth and survival.

Taken together, the potential target genes (i.e., KRAS, PIK3CA, TGF-β, HIF-1α and HNF-1β) confer a survival advantage against oxidative stress. Genome-scale reconstructions of metabolic, redox homeostasis, cell cycle, and survival signals are critical factors in disease progression (47,53). Accumulating evidence demonstrates that common metabolic reprogramming for cell survival, namely the Warburg effect, has been identified not only in cancer cells but also in endometriotic cells (20,44-46,53).

Functional roles of macrophages in the endometriotic/EAOC microenvironment. In response to inflammation, hypoxia, and oxidative stress, endometriosis creates a unique microenvironment composed of epithelium, stroma, mesenchyme, and infiltrative immune cells (62). Endometriotic cells adapt to and survive in hypoxic and oxidative stress conditions due to the high levels of hemoglobin, heme, iron, angiogenic factors (e.g., vascular endothelial growth factor), inflammatory cytokines and chemokines (e.g., TGF-β1, tumor necrosis factor alpha, interleukin-6 and -8, regulated on activation normal T cell expressed and secreted, and monocyte chemotactic protein-1 (62,63). These key mediators act as chemoattractants and enhance macrophage recruitment into endometriotic lesions. Iron-laden macrophages, especially M2 macrophages, are detected throughout the stroma of endometriotic cysts and CCC lesions (16,24,64). M2 macrophages scavenge iron and secrete antioxidants that relieve and eliminate excess oxidative stress in the surrounding environment (7). Heme oxygenase 1 (HO-1) is a key factor that contributes to antioxidant defenses (22). Co-culture experiments with macrophages and endometriotic cells have demonstrated that macrophage-derived HO-1 expression was induced by TGF-β1 produced by endometriotic cells (65). Addition of ROS to the co-culture system enhanced HO-1 production from macrophages (65). This crosstalk significantly enhances the antioxidant defense mechanism and protects endometriotic cells against oxidative injury, contributing to the progression of endometriosis (65). Moreover, a mouse model of endometriosis demonstrated that targeted depletion of macrophages blocks the growth of endometriotic lesions (66). Preclinical research suggests that endometriosis-macrophage crosstalk can provide a promising survival advantage to endometriotic cells (65,66).

Tumor-associated M2 macrophages contribute to tumor growth, invasion, metastasis, and treatment resistance in ovarian cancer (67); however, the effects of macrophages on the malignant transformation of endometriosis yielded inconsistent results. There were no statistically significant differences in the number of M2 macrophages in endometriotic cysts and CCC cells (24). In contrast, it was reported that the number of HO-1-positive M2 macrophages was significantly lower in the CCC group compared to the endometriotic cyst group (64). Additionally, CDC42-positive macrophages may prevent malignant transformation of endometriosis (68). CDC42, a small GTPase of the Rho-subfamily, regulates cell cycle progression. The number, polarization, and characteristics of infiltrating macrophages as well as their spatial distribution (e.g., closer proximity of M2 macrophages to target cells) play an important role in the carcinogenic process.

4. A promising tool for the early diagnosis of malignant transformation of endometriosis

Non-invasive diagnostic imaging. TVS is an effective imaging technique for the evaluation of ovarian masses, while magnetic resonance imaging may be used to identify TVS-indeterminate lesions. Increasing tumor size, rapidly growing mural nodules, papillary excrescences, or irregular and thick septations are features highly suspicious of malignancy (69). The conventional diagnostic modalities cannot distinguish benign from malignant lesions before the appearance of various morphological and anatomical changes in the cyst. Therefore, early-stage disease with little or no morphological changes may be missed during imaging surveillance. Histological examination is the gold standard for diagnosis, but it is an invasive procedure. Non-invasive diagnostic approaches to distinguish between endometriosis and EAOC are warranted. In 2008, Yamaguchi *et al* (4) showed for the first time that iron levels in endometriotic cysts are higher than in other benign ovarian cysts. Total iron, heme iron, and free iron levels in cystic fluids are useful markers in the differential diagnosis of endometriosis and EAOC (33). Total iron levels in patients with endometriosis were markedly higher than those with EAOC (median ± SD, 244.4±204.9 mg/l vs. 14.2±36.6 mg/l, $P<0.001$) (33). The total iron level that distinguishes endometriosis from EAOC provided 90.9% sensitivity and 100% specificity, with an optimal cut-off value of 64.8 mg/l (33). Recent advances in metallobiology have enabled the non-invasive quantification of iron levels. MR transverse relaxometry quantifies iron concentrations in endometriotic cysts using non-invasive techniques (70). MR relaxometry calculates the R2 value as a predicted value of iron concentration using a single-voxel multi-echo MR sequence (HISTO) by a 3T-MR system (70). R2 values highly correlated with iron concentrations, allowing for the rapid and non-invasive differentiation of endometriotic cysts from EAOC preoperatively, with a sensitivity of 86% and a specificity of 94% (70). MR relaxometry is a promising alternative for the early detection of malignant transformation of endometriosis (71-73). Additionally, real-time *in vivo* imaging methods used for the diagnosis of malignant transformation of endometriosis include electronic

absorption spectroscopy and near infrared approach in addition to MR transverse relaxometry (74). However, diagnostic imaging techniques other than MR relaxometry are not available in clinical practice. Endometriosis causes changes in biochemical markers, including iron, during malignant transformation; hence, novel diagnostic modalities may allow physicians to detect biochemical changes early in the disease before anatomical changes occur. The non-invasive quantification of iron levels will aid in the early diagnosis and provide information that improves disease management strategies.

Potential serodiagnostic biomarkers. The final part highlights new serum biomarkers that may distinguish between benign and malignant ovarian tumors and improve the diagnostic accuracy for ovarian cancer. CA-125 is widely used in the diagnosis and monitoring of patients with ovarian cancer (75). However, the use of CA-125 is limited by its low sensitivity for CCC and a high false positivity rate in endometriosis (76). As CCC is the prevalent type of EAOC in Japan, novel non-invasive biomarkers that can accurately distinguish CCC from endometriosis are urgently needed. Human epididymis protein 4 (HE4) levels are not elevated in benign diseases, such as endometriosis, and are not affected by the menstrual cycle or hormone therapy. HE4 also has higher specificity than CA-125 (0.93 vs. 0.75, respectively) (77,78). Hence, HE4 could be useful in distinguishing suspected malignant ovarian tumors from endometriosis. However, HE4 is affected by several factors, including menopausal status, age, smoking, and renal dysfunction (79). Serum HE4 levels were elevated in 90% of patients with high-grade serous ovarian cancer and 69% of patients with CCC tumors, indicating that HE4 is reliable in diagnosing serous ovarian cancer, while it seems to be a less useful marker for CCC (80). Moreover, scoring systems and prediction algorithms may better assess the risk for ovarian cancer (81,82). The Risk of Ovarian Malignancy Algorithm and Copenhagen Index, including CA-125, HE4, and menopausal status or age, have high diagnostic performance for differentiating benign from malignant lesions (81,82). Additionally, recent advances in secretome analysis and bioinformatics revealed a serine protease inhibitor, namely tissue factor pathway inhibitor 2 (TFPI2), as a potential biomarker for the detection of ovarian cancer, especially CCC (76,83). TFPI2 is elevated in the serum and tumor tissues of patients with CCC, suggesting that serum TFPI2 may be a clinically useful biomarker for diagnosing CCC (76,83). A prospective validation study revealed that TFPI2 provided sufficient specificity for predicting CCC (79.5%) (84). Additionally, CA-125 had a high false-positive rate (71.4% [15/21]) in endometriosis, whereas none of the patients with elevated CA-125 levels had elevated TFPI2 levels (84). Miyagi *et al* (84) evaluated the diagnostic value of CA-125 and TFPI2 for distinguishing endometriosis and CCC, demonstrating that TFPI2 is superior to CA-125 as a marker of CCC (AUC 0.855 vs. 0.520). Based on these results, TFPI2 testing became available for ovarian cancers under the national health insurance in Japan since April 2021. We believe that TFPI2 can be used as a reliable diagnostic and treatment marker for malignant transformation of endometriosis into CCC.

5. Discussion and future challenges

We discuss the current challenges and future directions for malignant transformation of endometriosis. Fig. 3 shows the redox balance and metabolic switch regulating the endometriotic cell fate (death or survival) during malignant transformation. Redox homeostasis is regulated in response to environmental changes. Hemoglobin, heme, and iron are abundant in endometriotic cysts and cause oxidative stress (5-7,22). In the oxidative microenvironment, endometriotic cells stimulate the production of HO-1 from the surrounding macrophages via TGF-β production (64,65). HO-1 is a key regulator of redox homeostasis that eliminates excessive ROS in endometriotic cells and prevents cell death (64,65). This crosstalk can help macrophages promote endometriotic cell survival. On the other hand, the response of endometriotic cells to harsh environmental conditions requires energy generated through a metabolic shift from aerobic glycolysis to mitochondrial oxidation. In endometriotic cells under excessive oxidative stress (a redox meter showing the red area in Fig. 3), a metabolic shift to oxidative phosphorylation ultimately results in the death of endometriotic cells via extra ROS production from mitochondria. Proper energy homeostasis is regulated by specific control systems (43,44). The PDK-PDH axis plays a key role in mitochondrial dynamics during energy switching (44,45). When redox homeostasis is balanced in an oxidative microenvironment (a meter showing the yellow area in Fig. 3), HIF-1α stimulates PDK activation via TGF-β1 expression. As a result, ROS accumulation is reduced by fermentative glycolysis through a metabolic shift from mitochondrial OXPHOS to aerobic glycolysis. Accumulation of ROS at sublethal doses may increase tumorigenic potential via DNA mutagenesis (29,30).

Recent genomic studies revealed that somatic mutations accumulated in cancer-related genes (e.g., ARID1A, PIK3CA, and KRAS proto-oncogenes) not only in epithelial cells of endometriosis but also in the normal endometrium, and that mutagenesis in a certain gene (i.e., apolipoprotein B mRNA Editing Enzyme Catalytic Subunit) is involved in the genomic heterogeneity of endometriosis (85). Candidate gene mutations may affect cell survival via changes in inflammation, redox homeostasis, and metabolism. Genetic diversity occurs over time (85). Endometriosis clones that have adapted to harsh environments have also fueled adaptation to a suitable environment for carcinogenesis. Since the ARID1A gene is mutated in 46-70% of patients with CCC (10), epigenetic inactivation of this gene also contributes to the development of CCC. Methemoglobin and oxyhemoglobin represent the major hemoglobin species of endometriosis and EAOC cystic fluid, respectively (27). Endometriotic cells surviving in oxyhemoglobin-rich antioxidant environments (a meter showing the green area in Fig. 3) have a greater ability for carcinogenesis possibly through (epi)genetic modulations of CCC susceptibility genes, leading to the initiation and progression of malignant transformation. Furthermore, some antioxidants (e.g., HO-1) produced by macrophages may play a role in tumor promotion and progression. Therefore, the main hallmarks of malignant transformation are the changes in redox signaling, energy metabolism, and tumor immune microenvironment. Understanding the molecular mechanisms

underlying these biological features will open new possibilities for the diagnosis, treatment, and management of the disease.

In clinical practice, conventional biomarkers and diagnostic imaging techniques are not sufficient for the early detection of malignant transformation of endometriosis. Novel imaging modalities (e.g., MR relaxometry) and biomarkers (e.g., TFPI2) are promising technologies. MR relaxometry offers the possibility of early diagnosis as changes in iron levels can be quantified quickly and non-invasively (70-73). Compared with conventional diagnostic imaging, MR relaxometry more accurately detects malignant transformation; however, there is limited data on its use. TFPI2 may become a useful biomarker for discriminating between endometriosis and CCC (76,83,84). In April 2021, TFPI2 testing was covered by the Japanese national health insurance system, enabling multicenter collaborative research to evaluate its diagnostic accuracy. Understanding the pathophysiology of malignant transformation may lead to the development of novel diagnostic imaging techniques and biomarkers. This review described the mechanisms of redox homeostasis, energy metabolism, and crosstalk with macrophages to better understand the malignant transformation of endometriosis and summarized the potential diagnostic and monitoring techniques for early diagnosis. Further studies are needed to determine how endometriotic cell clones that have acquired survival benefits undergo malignant transformation.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HK was in charge of conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, original draft writing, review and editing. Data authentication is not applicable. The author read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The author declares that they have no competing interests.

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