Lay Summary
The most commonly used medical therapies for endometriosis have contraceptives and other side effects associated with hormone suppression and are therefore unsuitable for women desiring pregnancy. One therapeutic strategy that may avoid hormone manipulation is focused on changing metabolic profiles that have been detected in cells/tissues from women with endometriosis. Endometriotic cells favor glycolytic metabolism over mitochondrial oxidative phosphorylation (OXPHOS) to produce essential energy for cell growth. Furthermore, the metabolic conversion from mitochondrial OXPHOS to aerobic glycolysis suppresses cell death through reduced generation of reactive oxygen species (ROS). This unique metabolic feature of endometriosis is important for cell survival and disease progression. Thus, changing the specific metabolic switch may increase mitochondrial ROS production, causing severe oxidative stress and cell death. This review describes new treatments by changing the metabolic profiles of endometriosis.
Nonhormonal therapy for endometriosis based on energy metabolism regulation

Hiroshi Kobayashi\textsuperscript{1,2}, Hiroshi Shigetomi\textsuperscript{1,3}, and Shogo Imanaka\textsuperscript{1,2).

\textsuperscript{1}Department of Obstetrics and Gynecology, Nara Medical University, Kashihara, 634-8522, Japan.
\textsuperscript{2}Department of Gynecology, Ms.Clinic MayOne, Kashihara, 634-0813, Japan.
\textsuperscript{3}Aska Ladies Clinic, Nara, 634-0001, Japan.

Running Title:
Nonhormonal therapy for endometriosis

Corresponding author:
Hiroshi Kobayashi, MD, PhD
Department of Obstetrics and Gynecology, Nara Medical University
840 Shijo-cho, Kashihara, 634-8522, Nara, Japan
Tel, +81-744-29-8877; Fax, +81-744-23-6557; Email, hirokoba@naramed-u.ac.jp
Abstract

Objectives: Ovarian function suppression is the current pharmacotherapy of endometriosis with limited benefit and adverse effects. New therapeutic strategies other than hormonal therapy are developed based on the molecular mechanisms involved in the hypoxic and oxidative stress environments and metabolism unique to endometriosis.

Methods: A literature search was performed between January 2000 and March 2021 in the PubMed database using a combination of specific terms.

Results: Endometriosis-associated metabolic changes have been organized into four hallmarks: (1) glucose uptake, (2) aerobic glycolysis, (3) lactate production and accumulation, and (4) metabolic conversion from mitochondrial oxidative phosphorylation (OXPHOS) to aerobic glycolysis. Endometriotic cells favor glycolytic metabolism over mitochondrial OXPHOS to produce essential energy for cell survival. Hypoxia, a common feature of the endometriosis environment, is a key player in this metabolic conversion, which may lead to glucose transporter overexpression, pyruvate dehydrogenase kinase 1 (PDK1) and lactate dehydrogenase kinase A (LDHA) activation, and pyruvate dehydrogenase complex inactivation. Evading mitochondrial OXPHOS mitigates excessive generation of reactive oxygen species (ROS) that may trigger cell death. Therefore, the coinactivation of LDHA and PDK1 can induce the accumulation of mitochondrial ROS by converting energy metabolism to mitochondrial OXPHOS, causing endometriotic cell death.

Conclusion: Metabolic pattern reconstruction in endometriotic lesions is a critical factor in cell survival and disease progression. One therapeutic strategy that may avoid hormone manipulation is focused on mitigating metabolic changes that have been detected in cells/tissues from women with endometriosis.

Key words: Endometriosis; Glycolysis; Hypoxia; Metabolism; Oxidative phosphorylation; Warburg effect

Introduction

Endometriosis is an estrogen-dependent, chronic inflammatory condition that contains tissue that resembles an endometrium with one or more of the following: stromal fibroblasts, epithelial cells, immune cells, and nerves and vascular/perivascular cells in sites outside the uterine cavity (Zondervan et al., 2020; Saunders and Horne, 2021). Moreover, it affects approximately 10% of all reproductive-aged women and is associated with pain and infertility (Hughes et al., 2015; Zondervan et al., 2020; Saunders and Horne, 2021). The treatment choice will depend on age at diagnosis, disease stage, the patient's symptoms, priorities and expectations, reproductive plans, safety, adverse effects incidence, tolerability, and cost (Ferrero et al., 2018). Medical endometriosis therapy should be considered pain symptom control and postoperative recurrence prevention within the framework of long-term therapeutic strategies (Ferrero et al., 2018). However, the available drugs (e.g., combined oral contraceptive pills, progestins, danazol, and gonadotropin-releasing hormone (GnRH) analogs) suppress ovarian function and are not curative (Hughes et al., 2015).
et al., 2015; Ferrero et al., 2018). Thus, patients with endometriosis urgently need long-term nonhormonal therapy without affecting fertility.

Endometriosis exists in a unique inflammatory microenvironment characterized by hormonal imbalance, hypoxia, and oxidative stress (McKinnon et al., 2016; Ito et al., 2017; Lin et al., 2018). Endometriotic cells undergo genetic, epigenetic, and metabolic alterations to overcome many obstacles (e.g., adaptation and survival to harsh environments, evasion of immune defenses, and invasion of adjacent tissues; Koninckx et al., 2019). These microenvironmental changes can enhance the survival of endometriotic cells through several main pathways (e.g., phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR), mitogen-activated protein kinases (MAPK; extracellular signal-regulated kinase (ERK)1/2, p38, and c-Jun NH2-terminal kinase (JNK)), and nuclear factor-kappaB (NF-κB) signaling pathways; McKinnon et al., 2016). These kinase pathways have been evaluated as effective targets for the treatment of other diseases, especially cancer (3), and can be potential candidates for personalized endometriosis therapy (McKinnon et al., 2016). However, the current generation of these targeted therapies can induce various adverse effects (McKinnon et al., 2016). Moreover, accumulating evidence shows that endometriotic cells may survive the hypoxic environment by upgrading their metabolic properties (Atkins et al., 2019). The metabolic shift between aerobic glycolysis and oxidative phosphorylation plays a major role in the development and progression of endometriosis, and the modification of their signaling pathways can be a viable target for therapeutic intervention (Liao et al., 2015; Kobayashi et al., 2021). The review aims to discuss the survival mechanism of endometriosis in hypoxic and oxidative stress environments and provide future perspectives on nonhormone treatment based on metabolic shifts.

Methods
Search strategy and selection criteria
A computerized literature search was performed to identify relevant studies reported in the English language. The PubMed electronic databases published between January 2000 and March 2021 were searched, combining the keywords endometriosis, hypoxia, oxidative stress, metabolism, glycolysis, oxidative phosphorylation, and Warburg. 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 the studies were synthesized using a descriptive review design with narrative methods. Figure 1 shows that the first identification phase includes the records identified through database search. Terms in the titles and abstracts were focused in the first screening stage. During the second screening phase, duplicates were removed, and titles, abstracts, and full-text articles were read to remove inappropriate papers. The final eligibility phase included the full-text articles for analysis after excluding those for which detailed data cannot be extracted.
Results and discussion
A review of the literature provides evidence that endometriotic cells may undergo metabolic change/adaptation to survive in extrauterine sites under conditions that may involve hypoxia and/or oxidative stress. The evidence is considered a shift in metabolic behavior under oxidative stress and hypoxia to inform the discussion of potential novel therapies. Here, three topics of endometriosis (i.e., Oxidative stress and redox imbalance, Hypoxic microenvironment, and metabolic reprogramming) will be discussed.

Oxidative stress and redox imbalance
Several theories have been proposed to explain the etiology of endometriosis, which includes the theories on retrograde menstruation, coelomic metaplasia, endometrial stem/progenitor cells, bone marrow stem cells, lymphatic and vascular spread, embryonic remnant differentiation or induction, and iatrogenic implantation (Zubrzycka et al., 2015). The most widely accepted is the retrograde menstruation theory. Blood containing endometrial cells is refluxed through the fallopian tubes during menstruation (Vinatier et al., 2000). Hemoglobin releases heme iron and free iron when red blood cells are hemolyzed in the peritoneal cavity or endometriotic cysts (Kobayashi et al., 2019). Hemoglobin generates superoxide radicals ($O_2^-$) when converted to methemoglobin via the autoxidation reaction (Iwabuchi et al., 2015). Free iron also generates hydroxyl radicals (OH$^-$), a powerful reactive oxygen species (ROS), through the Fenton reaction (Iwabuchi et al., 2015). Thus, endometriotic cells are always exposed to exogenous ROS, including superoxide anion, hydroxyl radical, and peroxynitrite (ONOO$^-$). High ROS levels induce oxidative DNA damage, methylation, and epigenetic errors (Menezo et al., 2016). Oxidative stress caused by ROS is a potential factor involved in the pathogenesis of endometriosis and may play a role in the onset and progression of this disease (Ito et al., 2017; Menezo et al., 2016). However, excessive ROS generation is also a key factor leading to cell death. Several studies have evaluated the oxidant–antioxidant balance in the blood, peritoneal fluid, follicular fluid, and tissue environment of patients with endometriosis (Santanam et al., 2002; Muscoli et al., 2003; Oner-Iyidoğan et al., 2004; Matos et al., 2009; Liu et al., 2013; Bamm et al., 2017; Chen et al., 2019). The ROS levels in both serum and follicular fluid of the endometriosis group were significantly higher than those in both serum and follicular fluid of the control group (Liu et al., 2013). The conjugated diene/triene, malondialdehyde, and oxidized low-density lipoproteins are lipid oxidation biomarkers (Santanam et al., 2002; Bamm et al., 2017). The levels of these lipid peroxidation end products were increased in both peritoneal fluid and serum of patients with endometriosis (Santanam et al., 2002; Bamm et al., 2017). Furthermore, the antioxidant capacities (e.g., superoxide dismutase (SOD) activity) were increased in endometriosis (Oner-Iyidoğan, 2004; Matos et al., 2009; Chen et al., 2019). ROS suppresses SOD production, but SOD expression is upregulated in endometriosis despite ROS overproduction (Muscoli et al., 2003). Antioxidants maintain cellular redox homeostasis by eliminating ROS and protect cells from ROS-induced damage (Chen et al., 2019). Thus, endometriotic cells can survive with oxidative stress exposure.
Hypoxic microenvironment

Endometrial fibroblasts are decidualized during pregnancy, allowing placenta formation and embryo implantation (Rytkönen et al., 2020). Placental tissue may have evolved mechanisms to tolerate hypoxic environments by expressing hypoxia-related genes such as hypoxia-inducible factor-1alpha (HIF-1α), vascular endothelial growth factor (VEGF), and transforming growth factor-beta1 (TGF-β1; Duzyj et al., 2018). Ectopic endometriotic cells also appear to inherit this property. Ectopic endometrial cells face severe hypoxic stress, but hypoxia plays a vital role in promoting pathological processes to facilitate endometriosis development (Lin et al., 2018; Lee et al., 2019; Wu et al., 2019). Under a hypoxic condition, cells undergo genetic and epigenetic modifications and evolve several survival processes, including steroidogenesis, inflammation, immune dysfunction, angiogenesis, epithelial–mesenchymal transition (EMT), and mesothelial–mesenchymal transition (MMT; Wu et al., 2019). The complex gene regulatory networks driven by the interplay between a hypoxic microenvironment and endometriotic cells allow endometriotic cells to survive (Wu et al., 2019). The effects induced by hypoxia are orchestrated by HIFs that regulate the expression of numerous genes, including VEGF, TGF-β1, PI3K/AKT, Wnt/β-catenin, and Notch (Laschke and Menger, 2012; Wilson, 2018; Rytkönen et al., 2020). Genes related to classical hypoxia pathways (e.g., HIF-1α, VEGF, and TGF-β1) have been extensively studied in endometriotic cells (Laschke and Menger, 2012; Rytkönen et al., 2020) and adjacent peritoneal mesothelial cells (Wilson, 2018). A hypoxic microenvironment stimulates endometriotic stromal cells (Dai et al., 2019) and peritoneal mesothelial cells (Lin et al., 2018) to produce and stabilize HIF-1α and promote the activation of TGF-β1/Smad and VEGF signal transduction pathways, contributing to increased cellular invasiveness, adhesiveness, cell survival, EMT, MMT, adhesion and fibrosis formation, and reduced apoptotic potential (Kasvandik et al., 2016). Endometriosis can be caused by local changes in tissues under the influence of oxidative stress and associated hypoxia. In addition, hypoxia has recently been emphasized to upregulate genes associated with glycolysis as described in the next section.

Metabolic reprogramming

The metabolic properties of endometriosis for energy acquisition are discussed in this section. In general, glycolytic conversion of glucose or fructose into adenosine 5′-triphosphate (ATP) generates energy to enable cell survival and growth (Figure 2). Cells utilize aerobic glycolysis to derive energy from the conversion of glucose to pyruvate and then lactate, regardless of oxygen availability (Vander Heiden et al., 2009). Aerobic glycolysis produces only two ATP per one glucose molecule, whereas additional 36 ATP molecules from one glucose molecule are produced through the tricarboxylic acid (TCA) cycle and the OXPHOS machinery (Vander Heiden et al., 2009). Aerobic glycolysis is an inefficient way to generate ATP, but it is a simple mechanism with a high ATP production rate. Aerobic glycolysis is activated by the stimulation of glycolytic enzymes such as glucose transporter (GLUT; McKinnon et al., 2014; Di Tucci et
al., 2018), phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3; Yi et al., 2019), pyruvate kinase M2 (PKM2; Tamada et al., 2012), pyruvate dehydrogenase kinase 1 (PDK1; Dunford et al., 2011), pyruvate dehydrogenase (PDH; Dunford et al., 2011), lactate dehydrogenase A (LDHA; Miao et al., 2013), and monocarboxylate transporter 1 (MCT-1; Halestrap, 2012; Figure 2; glycolytic pathways are surrounded by a yellow square). PFKFB3, as a key enzyme of glycolysis, positively regulates the glycolysis process (Yi et al., 2019). PKM2 is a final rate-limiting glycolysis enzyme and supports anabolic metabolism (Tamada et al., 2012). Pyruvate is converted to acetyl-coenzyme A (CoA), which is catalyzed by the PDH complex [36]. PDK1 is an enzyme that phosphorylates and deactivates PDH (Dunford et al., 2011). In addition, LDHA catalyzes the conversion of pyruvate to lactate and is considered a key checkpoint of anaerobic glycolysis (Miao et al., 2013). MCT-1 facilitates the rapid intracellular and extracellular transport of monocarboxylates (e.g., pyruvate, lactate, and the ketone bodies; Halestrap, 2012). Fatty acids are transported to the mitochondria and then metabolized to acetyl-CoA by β-oxidation, which feeds the TCA cycle. Acetyl-CoA is the key starting point of the mitochondrial TCA cycle and an essential fuel for ensuring OXPHOS (Figure 2; mitochondrial oxidative phosphorylation pathways are surrounded by a green square). However, stimulation of pyruvate flux into the mitochondrial oxidative metabolism increases ROS production, an inherent byproduct of oxidative metabolism, leading to impaired cell survival. Thus, a shift in metabolism from glycolysis to the TCA cycle/OXPHOS has not only the advantage of high energy production but also the drawback of ROS overproduction.

Endometriotic cells have been shown to reprogram metabolism pathways in response to various hypoxic and oxidative stress to fuel cell survival (Young et al., 2014, 2016; Dunford et al., 2011; Kasvandik et al., 2016; Lee et al., 2019). Endometriotic cells can induce metabolic conversion from oxidative phosphorylation to aerobic glycolysis to suppress ROS-mediated apoptosis. Four major steps are involved in cell metabolism: glucose uptake, glycolytic enzyme activation, lactate production and accumulation, and changes in mitochondrial function. For each step, the latest information on the metabolic alterations in endometriosis is summarized.

(1) Enhanced glucose uptake (Figure 2, yellow box)

Glycolysis begins with glucose uptake through solute carriers of the GLUT family (McKinnon et al., 2014). Solute carrier family 2 (SLC2A) gene encodes an integral plasma membrane glycoprotein, GLUT. The expression of SLC2A3 (GLUT3), SLC2A4 (GLUT4), and SLC2A5 (GLUT5) genes and proteins in endometriotic tissues was significantly higher than that in eutopic tissues (McKinnon et al., 2014). HIF1A gene expression was higher in endometriotic lesions than in a eutopic endometrium, and the HIF1A and SLC2A1 gene expression levels in the adjacent peritoneum of endometriotic lesions were higher than those in women without the disease (Di Tucci et al., 2018). An in vitro study showed that exposure of peritoneal mesothelial cells to TGF-β1 increased HIF1A and SLC2A1 mRNA expression (Di Tucci et al., 2018). Cellular glucose uptake by GLUTs is activated via the upregulation of TGF-β expression (McKinnon et al., 2018).
et al., 2014). Enhanced glucose uptake as a result of increased HIF-1 and TGF-β1 expression is a hallmark of endometriosis. Therefore, endometriosis causes metabolic reprogramming by increasing glucose uptake via the GLUT family.

(2) Increased glycolytic capacity and lactate production (Figure 2, yellow box)

Ectopic endometriotic cells exhibit more hypoxia than their eutopic counterparts (Lee et al., 2019). Some researchers compared tissue, peritoneal fluid, follicular fluid, and blood samples from patients with endometriosis to controls and showed significant changes in glycolytic pathway-specific genes and their transcripts (HIF-1, TGF-β, LDHA, PDK1, PDH, and SOD) and metabolites (glucose and lactate), indicating a distinct glucose metabolic signature (Young et al., 2014, 2016; Qi et al., 2014; Marianna et al., 2017; Horne et al., 2019). Lactate, an essential glycolysis product, is a major metabolic fuel, energy source, and gluconeogenic precursor. Lactate concentration was positively correlated with TGF-β1 in peritoneal fluid, and both of which were significantly higher in women with endometriosis than in women without endometriosis (Young et al., 2014, 2016; Qi et al., 2014; Horne et al., 2019). TGF-β1 can induce the metabolic conversion of glucose to lactate in the endometriotic lesions and adjacent peritoneum, possibly through hypoxia-induced HIF-1α expression (Young et al., 2014, 2016). Moreover, hypoxia-induced PDK1 upregulation and increased lactate production and oxygen consumption rate in ectopic endometrial stromal cells compared to normal endometrial stromal cells (Lee et al., 2019). This is thought to be because PDK1 suppressed the conversion of pyruvate to acetyl-CoA through the inhibition of PDH activity (Dunford et al., 2011). Exposure of mesothelial cells to TGF-β1 increased the production of mRNAs encoded by glycolysis-associated genes, namely, PDK1 and LDHA (Young et al., 2014). Glycolysis-related gene LDHA was more highly expressed in endometriotic lesions than in a eutopic endometrium (Young et al., 2014). Furthermore, follicular fluid in patients with endometriosis had lower glucose levels and higher levels of lactate, pyruvate, and VEGF than those in follicular fluid in control participants (Marianna et al., 2017; Pocate-Cheriet et al., 2020). Increased glucose uptake and consumption and accumulation of lactate were common features of endometriotic cells (Qi et al., 2014). Lactate has been reported to be proangiogenic (Hunt et al., 2008). Although no experimental data using endometriotic cells exist, lactate stimulates VEGF production by tumor and endothelial cells, leading to enhanced migration and resulting in lactate-induced angiogenesis (Hirschhaeuser et al., 2011; Marianna et al., 2017). Altogether, endometriotic cells have an increased glycolytic flux, which depends on the overexpression of glycolysis-related genes or their transcripts (HIF-1α, TGF-β, GLUT, LDHA, and PDK1), resulting in lactate overproduction and accumulation (Young et al., 2014, 2016; Qi et al., 2014; Marianna et al., 2017; Horne et al., 2019). The metabolic switch of increased glycolysis in endometriosis is thought to be driven primarily by TGF-β and HIF-1α.

(3) Metabolic conversion from TCA cycle/OXPHOS to aerobic glycolysis (Figure 2, green box)
Activation of aerobic glycolysis raises two possibilities. First, pyruvate is channeled into the mitochondria and converted to acetyl-CoA, and then enters the TCA cycle. Hypoxia-induced PDK1 expression results in decreased PDH activity, suppresses the conversion of pyruvate to acetyl-CoA, and accumulates pyruvate (Dunford et al., 2011; Young et al., 2014, 2016; Kasvandik et al., 2016; Lee et al., 2019). Second, LDHA promotes the conversion of pyruvate to lactate and suppresses the production of acetyl-CoA. Therefore, the conversion of pyruvate to acetyl-CoA in endometriosis may be suppressed by increased LDHA and PDK1 activity and decreased PDH activity (Young et al., 2014, 2016; Kasvandik et al., 2016).

Next, reports on the concentrations of intermediate metabolites involved in glycolysis and the TCA cycle from body fluid samples in patients with endometriosis and controls were summarized. Endometriosis patients showed greater changes in levels of metabolites (e.g., glucose, lactate, citrate, alpha-ketoglutarate, succinate, and malate) compared to controls. Serum (Dutta et al., 2012) and follicular fluid (Marianna et al., 2017; Karaer et al., 2019) samples from patients with endometriosis showed elevated lactate and succinate levels and reduced glucose levels compared to controls. Metabolomics analysis revealed that citrate, alpha-ketoglutarate (α-KG), and succinate were elevated in endometriosis (Jana et al., 2013) whereas malate was decreased (Atkins et al., 2019). The cause for the elevated citrate, α-KG, and succinate in endometriosis was considered. In general, glycolysis, glutaminolysis, or fatty acid β-oxidation provides the energy and macromolecules required for cell survival. For example, cancer patients show distinctly altered metabolism involved in glycolysis, TCA cycle, glutaminolysis, and fatty acid metabolism (Zhu et al., 2017). In the event of an energy crisis, the glutaminolysis involved in the conversion of glutamine to α-KG is activated to sustain energy metabolism (DeBerardinis et al., 2007). Glutaminolysis stimulates a pathway in which citrate was formed from α-KG through reductive carboxylation of isocitrate dehydrogenase (Wise et al., 2011). Therefore, endometriotic mitochondria can produce large amounts of α-KG and citrate (Figure 2, green box). Furthermore, glutaminolysis supports the production of glutathione, a major player in maintaining redox homeostasis (Wise et al., 2010). Thus, endometriosis can adapt to a unique environment by suppressing oxidative stress and enhancing its antioxidant capacity.

Surprisingly, despite survival in harsh environments, mitochondrial energy production and metabolism are reduced in endometriotic tissue compared to normal endometrial tissue (Atkins et al., 2019). Peritoneal mesothelial cells adjacent to endometriotic lesions also exhibited significantly higher glycolysis, increased lactate production, and lower mitochondrial respiration compared to those from women without the disease (Horne et al., 2019). These data suggest that endometriotic cells and adjacent peritoneal mesothelial cells are characterized by TCA cycle/OXPHOS arrest and metabolic shift to aerobic glycolysis. Endometriosis can alter cellular metabolism and can strategically reduce energy production to avoid excessive mitochondrial ROS production. The electron-producing oxidative pathway appears to be stopped in endometriosis, resulting in the lack of energy production.

Does this suggest mitochondrial dysfunction? The metabolic shift from OXPHOS to glycolysis
is known as the Warburg effect and is a characteristic of many cancers (Kasvandik et al., 2016; Liberti and Locasale, 2016; Atas et al., 2020). This mechanism can be driven by the TGF-β1–HIF-1α–PDK–PDH–LDHA system (Kim et al., 2006; Young et al., 2016; Wang et al., 2019; Atas et al., 2020). HIF-1 and TGF-β increased LDHA expression, promoted lactate production from pyruvate, and inhibited acetyl-CoA production from pyruvate through PDH deactivated by PDK1, consequently reducing mitochondrial energy production and ROS generation (Liao et al., 2015; Wang et al., 2019). The advantage of the Warburg effect is to suppress ROS overproduction, activate the survival signal of endometriotic cells, and thus prevent cell death (Kobayashi et al., 2021). Like cancer cells, endometrial cells may shift energy metabolism from OXPHOS to aerobic glycolysis, suppress ROS production, and then promote survival (Liao et al., 2015; Kobayashi et al., 2021). Alterations in the metabolic phenotype of endometriotic cells and adjacent peritoneal mesothelial cells are considered adaptations of endometriosis to the microenvironment rather than mitochondrial dysfunction.

Nonhormonal endometriosis treatment
This section discusses therapies that may alter energy metabolism, including the so-called Warburg effect. Treatment strategies for endometriosis have been divided into three categories: (1) glucose uptake suppression, (2) aerobic glycolysis suppression, and (3) metabolic switch from aerobic glycolysis to OXPHOS. Not all of the drugs described below have yielded promising preclinical outcomes for endometriosis. Some drugs that have been tested as therapies in preclinical models involving cancer cells that also exhibit altered metabolism have been considered (Table 1).

(1) Glucose uptake suppression
The potent GLUT inhibitors can attenuate glycolysis and suppress the growth of various cancer cells (Reckzeh et al., 2019). GLUT and SGLT inhibitors include genistein, phlorizin, ritonavir, indinavir, STF-31, and WZB117 (Jodeleit et al., 2018). Genistein downregulates HIF-1α, inactivating GLUT1 and HK2 to suppress aerobic glycolysis (Li et al., 2017). GLUTs were identified as off-target molecules of the HIV protease inhibitor ritonavir (Jodeleit et al., 2018). They exert antitumor effects by targeting GLUT1 via inhibiting glucose uptake in tumor cells. Recently, the glucose uptake inhibitors, which target GLUT isoforms, have also been studied for endometriotic cells. In particular, GLUT inhibitors may be an attractive target for the nonhormone-based treatment of endometriosis (McKinnon et al., 2014). The mRNA levels of GLUT1/3 and MCT1/4 were decreased in atorvastatin and resveratrol sole and simultaneous-treated groups in experimental endometriosis models (Bahrami et al., 2021). Atorvastatin did not cause significant changes during the glucose tolerance test, but coadministration of atorvastatin and resveratrol suppressed glycolysis and neovascularization (Bahrami et al., 2021). The simultaneous administration of atorvastatin and resveratrol can inhibit endometriosis development (Bahrami et al., 2021). Gui-Zhi-Fu-Ling-capsules, a classic Chinese medicinal formula, may have benefits in inhibiting endometriosis development through the
suppression of the expression levels of TGF-β1, GLUT4, and VEGF in a rat endometriosis model (Zhou et al., 2018). Thus, inhibition of glucose uptake may be promising therapeutic targets for endometriosis (McKinnon et al., 2014).

(2) Aerobic glycolysis suppression

Glycolytic enzyme inhibitors such as hexokinase (HK), phosphofructokinase (PHK), and PKM2 have been preclinically studied in cancer treatment.

**HK:** Hexokinase, an exose-phosphorylating enzyme for aerobic glycolysis, is overexpressed in many tumor cells (Ciscato et al., 2021). Treatments with 2-deoxy-D-glucose, 3-bromopyruvate, or lonidamine inhibit the key enzyme hexokinase of glycolysis, and genetic ablation of hexokinase 2 inhibits tumor growth in mouse models (Ciscato et al., 2021).

**PFK:** Phosphofructokinase-1 (PFK1), a primary glycolysis enzyme, is involved in the conversion of fructose-6-phosphate to fructose 1,6-bisphosphate (Li et al., 2017). (E)-1-(pyridin-4-yl)-3-(quinolin-2-yl)prop-2-en-1-one (PFK15) was developed as a selective antagonist of PFK–PFKFB3 (Li et al., 2017). PFK15 has been demonstrated to be effective in treating head and neck squamous cell carcinoma in xenograft mouse models (Li et al., 2017). Furthermore, the PFKFB3 expression in endometriotic cells is known to be upregulated by heat shock factor 1 (HSF1; Wang et al., 2021). In addition, Wang et al. (2021) showed that the HSF1 inhibitor KRIIB11 suppressed endometriosis progression in a mouse model.

**PKM2:** The M2 splice isoform of PKM2 eventually produces pyruvate and releases energy. High PKM2 activity is associated with glycolytic capacity and tumor growth and metastasis (Zhou et al., 2020). Suppression of PKM2 expression attenuated cancer cell growth via modulating immunometabolism (Zhou et al., 2020).

Suppression of the aerobic glycolytic pathway may become a new target for endometriosis treatment, but studies are still in their infancy (Wang et al., 2021).

(3) Metabolic switch from aerobic glycolysis to OXPHOS

The reversal of metabolism from OXPHOS to glycolysis, a metabolic characteristic of cancer cells, may be a therapeutic strategy that induces cell death through ROS overproduction by activating mitochondrial energy metabolism. The conversion of pyruvate to acetyl-CoA needs to be accelerated to reach that goal. PDH is essential for shuttling pyruvate into the mitochondria and fueling the TCA cycle. PDH activity is inhibited by PDK, and PDK inhibitors may help in activating PDH enzymatic activity. In addition, dichloroacetate (DCA) is a small-molecule pyruvate-mimetic PDK inhibitor (Bonnet et al., 2007). DCA promotes oxidative metabolism from anaerobic glycolysis to mitochondrial OXPHOS through PDH activation by PDK1 inhibition (Sun et al., 2010; Horne et al., 2019; Tataranni and Piccoli, 2019). This drug was shown to reverse the PDK-induced glycolytic phenotype (Bonnet et al., 2007; Tataranni and Piccoli, 2019). DCA suppressed the growth of some tumors in the field of cancer, and several preclinical studies
have been reported (Bonnet et al., 2007; Sun et al., 2010; Sanchez et al., 2013; Tataranni and Piccoli, 2019; Korga et al., 2019). DCA can induce cell death via excess ROS produced by OXPHOS (Tataranni and Piccoli, 2019). Therefore, this drug is a promising adjuvant chemotherapeutic agent as an oxidative stress enhancer (Korga et al., 2019). For example, DCA is potentially effective against multiple myeloma in animal models (Sanchez et al., 2013). In line with this theory, novel clinical DCA studies in cancer therapy are underway (Chu et al., 2015; Garon et al., 2014; Tian et al., 2019). The phase 1 study evaluated the safety, tolerability, recommended dose, pharmacokinetics, and pharmacodynamics of oral DCA in patients with advanced solid tumors (Chu et al., 2015). DCA produced side effects, including neurotoxicity. The open-label phase II trial determined the response rate, safety, and tolerability of oral DCA in patients with metastatic breast cancer and advanced-stage nonsmall cell lung cancer (Garon et al., 2014). However, oral DCA did not confer a clinical benefit in patients with previously treated advanced cancer. In addition, the pharmacokinetic profile for DCA varied from patient to patient, and the overall response rate was low in patients with multiple myeloma (Tian et al., 2019). PDK is a druggable target and may pave the way for further approaches to cancer.

Preclinical studies have shown that selective PDK inhibition suppresses the progression of endometriosis in animal models (Lee et al., 2019; Horne et al., 2019). In vitro and in vivo studies showed that DCA reduced lactate secretion and suppressed endometrial stromal cell proliferation in coculture experiments with endometrial stromal cells and peritoneal mesothelial cells (Horne et al., 2019). In addition, DCA decreased the oxygen consumption rate of ectopic endometrial stromal cells (Lee et al., 2019). Oral DCA administration decreased peritoneal fluid lactate concentration and lesion size in a mouse model of experimental endometriosis (Horne et al., 2019). Aerobic glycolysis mediates growth promotion and resistance to apoptosis of endometriotic cells, and a metabolic shift from glycolysis to OXPHOS is considered a promising therapeutic endometriosis strategy (Kim et al., 2021). A single-arm study has begun to determine whether DCA therapy is an effective and acceptable treatment for endometriosis-related pain (Leow et al., 2021). This study provides a rationale for targeting metabolic shifts as a nonhormonal therapy for women with endometriosis.

In addition, some reports on PDK1 inhibitors such as ilimaquinone (IQ; Kwak et al., 2020) and Caesalpinia sappan L. (Kim et al., 2021) exist. IQ is a sesquiterpene quinone isolated from the marine sponge Smenospongia cerebriformis and inhibits PDK1 activity in cancer cells (Kwak et al., 2020). Moreover, C. sappan is an herbal medicinal product used to treat algomenorrhea and amenorrhea (Kim et al., 2021). Furthermore, C. sappan suppresses PDK1 expression and increases mitochondrial ROS levels, which, in turn, promotes endometrial cell apoptosis (Kim et al., 2021).

Another candidate drug is the LDHA inhibitors. However, this drug has never been used to treat endometriosis in preclinical studies. In light of previous reports, it can be speculated that the coinactivation of LDHA and PDK1 functions shifts from aerobic glycolysis to the TCA cycle/OXPHOS, causing ROS overproduction and culminating in cell death. LDHA regulates pyruvate production and thus acts as a link.
between glycolysis and the TCA cycle/OXPHOS (Miao et al., 2013). LDHA is elevated in many cancer types. Inhibition of LDHA activity, either by RNA interference or by pharmacological inhibitors, can block tumor growth and progression in vitro and in vivo (Oermann et al., 2012; Miao et al., 2013). Specific LDHA inhibitors include a small-molecule inhibitor 3-dihydroxy-6-methyl-7-(phenylmethyl)-4-propynaphthalene-1-carboxylic acid (FX11; Le et al., 2010), N-hydroxy-2-carboxy-substituted indole compounds (Granchi et al., 2011), and epigallocatechin (Wang et al., 2013). LDHA siRNA or FX11 can effectively inhibit cancer growth through the shift to OXPHOS and increased intracellular ROS (Arseneault et al., 2013). From the aforementioned data, inactivating LDHA is possible to inhibit the endometriotic cell growth possibly through ROS overproduction. While revising this manuscript, an interesting paper was reported. The silencing of the LDHA expression in immortalized cells promotes apoptosis through glycolysis inhibition and mitochondrial function suppression (Zheng et al., 2021). Future studies are expected to verify the effectiveness of the combination treatment of DCA and LDHA inhibitors in endometriosis. A therapeutic strategy focusing on the shift from aerobic glycolysis to the TCA cycle/OXPHOS may be a promising nonhormonal therapy for endometriosis.

Endometriotic cells are constantly exposed to iron-derived oxidative stress and hypoxic condition, and the shift from aerobic glycolysis to the TCA cycle/OXPHOS may cause ROS overproduction, leading to cell death. Increases in glucose uptake, glycolytic reserve, and gene expression of glycolytic enzymes (HK2, PFKFB3, PKM2, LDHA, and PDK1) are associated with a compensatory decrease in mitochondrial respiration. Molecules that are directly involved in the reprogramming of mitochondrial metabolism may be therapeutic targets for endometriosis (Kobayashi et al., 2021). In particular, the metabolic shift may be an attractive target for nonhormone-based endometriosis treatment. Treatment strategies that utilize metabolic reprogramming are implemented not only in cancer but also in sepsis (Bakalov et al., 2020), schizophrenia (Pruett and Meador-Woodruff, 2020), autoimmune disease (Kornberg, 2020), or mitochondrial disease (Kobayashi et al., 2021).

Summary and conclusions
The currently available treatment options for endometriosis suppress ovarian function, but no cure currently exists. Such treatment is unsuitable for women desiring pregnancy. Therefore, studies on new drugs that do not suppress ovarian function have commenced. Several researchers focused on genes and proteins that may affect metabolic pathways to promote endometriotic cell survival and growth. The metabolism characteristic of endometriosis is significantly affected by estrogen (Kobayashi et al., 2021). Moreover, estrogen is involved not only in hormonal action but also in various functions (e.g., mitochondrial biosynthesis and energy metabolism). Estrogen can also affect ATP production, energy conversion, ROS production, and antioxidant defense through the regulation of mitochondrial gene expression. Estrogen downstream target genes (e.g., peroxisome proliferator-activated receptor-gamma coactivator 1α), involved in mitochondrial metabolic biosynthesis, may be potential targets for nonhormonal therapy for
endometriosis (Kobayashi et al., 2021). Basic and preclinical studies are steadily progressing, although these drugs are still far from clinical application.

Endometriotic cells often reprogram their metabolic pathways to adapt to environmental challenges and facilitate survival. Endometriotic cells are essentially exposed to a hypoxic microenvironment. HIF-1- and TGF-β-mediated upregulation of LDHA and PDK1 expression induced by hypoxia and oxidative stress is an adaptive phenomenon in endometriosis (Young et al., 2014, 2016; Qi et al., 2014; Marianna et al., 2017; Horne et al., 2019). The actual balance between glycolysis and the TCA cycle/OXPHOS is regulated by glycolytic predominance (Young et al., 2014; Marianna et al., 2017; Lee et al., 2019; Wang et al., 2019; Reckzeh et al., 2019). This is supported by measurements showing local elevation of HIF-1, TGF-β, PDK1, LDHA, and lactate as well as the counterclockwise rotation of the TCA cycle (i.e., elevated levels of citrate, α-KG, and succinate; Dutta et al., 2012; Marianna et al., 2017; Karaer et al., 2019; Figure 2). Metabolic changes in endometriosis shift from the TCA cycle/OXPHOS to aerobic glycolysis and suppress ROS overproduction for its survival.

This phenomenon is similar to the Warburg effect in cancer (Kasvandik et al., 2016; Liberti and Locasale, 2016; Atas et al., 2020). Oxidative stress and hypoxia are largely involved in the development and progression of endometriosis, and functional modifications of these signaling pathways may be a viable target for endometriosis treatment (Kasvandik et al., 2016). Negative regulation of the Warburg effect can increase endogenous ROS and then induce endometriotic cell death (Lim et al., 2021). Therefore, inhibition of PDK and LDHA may be a new strategy in nonhormonal therapy for endometriosis. Currently, a few small-molecule inhibitors and natural compounds have been reported to inhibit PDK (Anwar et al., 2021) or LDHA (Wang et al., 2013) with promising oral administration (Table 1).

In conclusion, metabolic flexibility in endometriosis is the ability to adapt to environmental changes. The reverse Warburg effect could be an attractive target for developing nonhormonal treatments for endometriosis.

Declaration of interest
The author declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Funding
This work was supported by the Japan Society for the Promotion of Science (JSPS), Grant Number JP16K11150, 18K09269 and 18K09234.

Contributions
Hiroshi Kobayashi (HK) performed the literature search and collected data using the Web database. HK
made a contribution to conception of the study and also contributed to the interpretation of included research studies. The final version of the manuscript has been read and approved by HK.

Acknowledgements
Not applicable.

References


Deck CA, Anderson WG, Conlon JM & Walsh PJ 2017 The activity of the rectal gland of the North Pacific spiny dogfish Squalus suckleyi is glucose dependent and stimulated by glucagon-like peptide-1. *Journal of Comparative Physiology B* 187 1155-1161.


Halestrap AP 2012 The monocarboxylate transporter family--Structure and functional characterization.


Copyright © 2021 the authors

Downloaded from Bioscientifica.com at 11/28/2021 05:15:26AM
via free access


Oner-Iyidoğan Y, Koçak H, Gürdöl F, Korkmaz D & Buyru F 2004 Indices of oxidative stress in eutopic
and ectopic endometria of women with endometriosis. Gynecologic and Obstetric Investigation 57 214-217.


Pruett BS & Meador-Woodruff JH 2020 Evidence for altered energy metabolism, increased lactate, and decreased pH in schizophrenia brain: A focused review and meta-analysis of human postmortem and magnetic resonance spectroscopy studies. Schizophrenia Research 223 29-42.


Vander Heiden MG, Cantley LC & Thompson CB 2009 Understanding the Warburg effect: the metabolic
requirements of cell proliferation. *Science* 324 1029-1033.


Wang Y, Xiu J, Yang T, Ren C & Yu Z 2021 HSF1 promotes endometriosis development and glycolysis by up-regulating PFKFB3 expression. *Reproductive Biology and Endocrinology* 19 86.


Table 1. Summary of the drugs tested in endometriosis and other models
The table includes target protein/metabolite, mechanism of action, in vitro/in vivo/animal experiments, results and references. The results of preclinical studies on endometriosis are shown in yellow.

Figure 1. The number of articles identified by searching for keyword combinations.
This figure shows the number of articles identified by keyword 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, hypoxia; 3, oxidative stress; 4, metabolism; 5, glycolysis; 6, oxidative phosphorylation; and 7, Warburg.

Figure 2. Glycolysis and mitochondrial metabolism in endometriosis.
Colored boxes indicate major metabolic pathways: aerobic glycolysis (yellow box) and the TCA cycle/OXPHOS (green box). Red letters indicate increased genes, gene transcripts, enzymes, and metabolites; blue letters indicate reduced expression.
<table>
<thead>
<tr>
<th>Target protein / metabolite</th>
<th>The mechanism of action</th>
<th>In vitro / in vivo / animal experiments</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Suppression of glucose uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC2A*</td>
<td>Glucose transporter</td>
<td>Human tissue samples</td>
<td>Glucose transporter SLC2A expression in ectopic endometriotic lesions is significantly higher than in eutopic endometrial tissue.</td>
<td>McKinnon et al. (2014)</td>
</tr>
<tr>
<td>GZFLC*</td>
<td>A classic Chinese medicinal formula</td>
<td>Rat endometriosis model</td>
<td>GZFLC suppressed the expression levels of TGF-β1, GLUT4, and VEGF, and inhibited the development of endometriosis.</td>
<td>Zhou et al. (2018)</td>
</tr>
<tr>
<td>Atorvastatin and resveratrol*</td>
<td>Statin: inhibitors of hydroxymethylglutaryl-CoA reductase</td>
<td>Female Wistar rats / the experimental endometriosis</td>
<td>Effects of atorvastatin and resveratrol against the experimental endometriosis; evidence for glucose and monocarboxylate transporters.</td>
<td>Bahrami et al. (2021)</td>
</tr>
<tr>
<td>(2) Suppression of aerobic glycolysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genetic ablation HK2</td>
<td>A family of ubiquitous exose-phosphorylating enzymes that prime glucose for intracellular utilization.</td>
<td>Mouse models; Hepatocellular carcinoma / Colorectal cancer / Glioblastoma etc.</td>
<td>Genetic ablation of HK2 inhibited tumor growth.</td>
<td>Ciscato et al. (2021)</td>
</tr>
<tr>
<td>(E)-1-(pyridin-4-yl)-3-(quinolin-2-yl)prop-2-en-1-one (PFK15)</td>
<td>Enzymes related to glycolysis; Inhibitors of PFKFB3; Glycolysis blockage by targeting PFKFB3.</td>
<td>In vitro / in vivo / mouse models; Head and neck squamous cell carcinoma.</td>
<td>Targeting aerobic glycolysis with PFKFB3 inhibitors suppressed tumor growth and metastasis, providing a promising strategy for cancer treatment.</td>
<td>Li et al. (2017)</td>
</tr>
<tr>
<td>Benserazide: Inhibitors of PKM2</td>
<td>PKM2 is an enzyme that generates pyruvate and ATP in the glycolytic pathway.</td>
<td>In vitro / in vivo; Melanoma</td>
<td>Benserazide blocked PKM2 enzyme activity, leading to inhibition of aerobic glycolysis; Benserazide inhibited tumor cell proliferation, colony formation, invasion and migration in vitro and in vivo models.</td>
<td>Zhou et al. (2020)</td>
</tr>
<tr>
<td>*Inhibitor of HSF1: KRIIB11</td>
<td>A transcription factor that is rapidly induced after temperature stress and binds heat shock promoter elements</td>
<td>In vitro / in vivo / mouse models; Endometriotic epithelial cell line (11Z) and human endometrial stromal cell line (ESC).</td>
<td>HSF1 promoted endometriosis development and glycolysis by up-regulating PFKFB3 expression; The HSF1 inhibitor KRIIB11 abrogated endometriosis progression in vitro and in vivo.</td>
<td>Wang et al. (2021)</td>
</tr>
<tr>
<td>(3) Metabolic switch from aerobic glycolysis to OXPHOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DCA</strong></td>
<td>**DCA is an anti-cancer agent that can</td>
<td><strong>Several cancers</strong></td>
<td>**DCA inhibits mitochondrial PDK, shifted</td>
<td><strong>Bonnet et al.</strong></td>
</tr>
<tr>
<td></td>
<td>reverse the glycolytic phenotype in cancer cells; A pyruvate analogue; A prototypical PDK inhibitor.</td>
<td></td>
<td>metabolism from glycolysis to glucose oxidation, decreased mitochondrial membrane potential, and increased mitochondrial H$_2$O$_2$; DCA decreased proliferation, induced apoptosis, and inhibited tumor growth; The orally available DCA is a promising selective anticancer agent.</td>
<td>(2007)</td>
</tr>
<tr>
<td><strong>DCA</strong></td>
<td></td>
<td></td>
<td><strong>DCA has antiproliferative properties in addition to promoting apoptosis.</strong></td>
<td>Sun et al. (2010)</td>
</tr>
<tr>
<td><strong>DCA</strong></td>
<td><strong>In vitro / in vivo / mouse models; multiple myeloma</strong></td>
<td><strong>DCA may be effective in multiple myeloma patients with an activated aerobic glycolytic pathway.</strong></td>
<td>Sun et al. (2013)</td>
<td></td>
</tr>
<tr>
<td><strong>DCA</strong></td>
<td><strong>Several cancer models; Clinical administration in cancer therapy</strong></td>
<td><strong>Coadministration of DCA with conventional chemotherapy, radiotherapy, other drugs, or natural compounds may be promising for effective cancer therapy.</strong></td>
<td>Tataranni &amp; Piccoli (2019)</td>
<td></td>
</tr>
<tr>
<td>Three glycolysis inhibitors: DCA, 2-deoxyglucose, or 3-promopyruvate.</td>
<td><strong>In vitro; Hepatocellular carcinoma HepG2 cells</strong></td>
<td><strong>The chemotherapeutic agent and glycolysis inhibitors induced oxidative stress-associated damage in HepG2 cells.</strong></td>
<td>Korga et al. (2019)</td>
<td></td>
</tr>
<tr>
<td><strong>DCA</strong></td>
<td><strong>A phase 1 study in patients with advanced solid tumors</strong></td>
<td><strong>The phase 1 study was undertaken to assess the safety, recommended dose, and pharmacokinetic profile of oral DCA in patients with advanced solid tumors.</strong></td>
<td>Chu et al. (2015)</td>
<td></td>
</tr>
<tr>
<td><strong>DCA</strong></td>
<td><strong>An open label phase II trial</strong></td>
<td><strong>The clinical trial determined the response rate, safety, and tolerability of oral DCA in patients with metastatic breast cancer and advanced stage non-small cell lung cancer. Patients with previously treated advanced cancer did not benefit from oral DCA.</strong></td>
<td>Garon et al. (2014)</td>
<td></td>
</tr>
<tr>
<td><strong>DCA</strong></td>
<td><strong>A pilot phase 2 study in patients with multiple myeloma</strong></td>
<td><strong>The pharmacokinetic profile for DCA varied from patient to patient, and the overall response rate for multiple myeloma was low.</strong></td>
<td>Tian et al. (2019)</td>
<td></td>
</tr>
<tr>
<td><strong>DCA</strong></td>
<td><strong>In vitro. ectopic endometriotic stromal cells</strong></td>
<td><strong>The PDK1 expression was upregulated in ectopic stromal cells through hypoxia-induced signals; Inhibition of PDK1 activity by treatment with DCA induced ectopic stromal cell death.</strong></td>
<td>Lee et al. (2019)</td>
<td></td>
</tr>
<tr>
<td><strong>DCA</strong>*</td>
<td>In vitro / in vivo / mouse models; Endometriosis.</td>
<td>Human peritoneal mesothelial cells (HPMC) in women with endometriosis exhibited metabolic conversion from OXPHOS to aerobic glycolysis due to reduced enzymatic activity of PDH compared to HPMC in disease-free women; TGF-β1 is believed to be responsible for this abnormal phenotype; Treatment of endometriosis HPMC with DCA normalizes metabolism and suppresses the proliferation of endometrial stromal cells; Oral DCA reduced endometriosis lesion size in a mouse model.</td>
<td>Horne et al. (2019)</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td><strong>DCA</strong></td>
<td>Sepsis model: Drosophila melanogaster model of surviving sepsis infected with Staphylococcus aureus.</td>
<td>DCA treatment was associated with improved lifespan of sepsis survivors.</td>
<td>Bakalov et al. (2020)</td>
<td></td>
</tr>
<tr>
<td><strong>IQ</strong></td>
<td>A sesquiterpene quinone isolated from the marine sponge <em>Smenospongia cerebriformis</em>; PDK1 inhibitor</td>
<td>Human and murine cancer cells, such as A549, DLD-1, RKO, and LLC cells.</td>
<td>A novel candidate for anticancer therapeutics that act via the inhibition of PDK1 activity.</td>
<td>Kwak et al. (2020)</td>
</tr>
<tr>
<td><strong>Caesalpinia sappan L. (family Leguminosae)</strong>*</td>
<td>A herbal medicinal product used to treat gynecological symptoms, including amenorrhea. PDK1 inhibitor</td>
<td>In vitro; endometriotic cells</td>
<td>Caesalpinia sappan inhibited lactate production and phosphorylation of PDH by reducing the expression of PDK1; A novel drug candidate for treating endometriosis by inhibiting aerobic glycolysis and inducing ROS-mitochondria-mediated apoptotic cell death.</td>
<td>Kim et al. (2021)</td>
</tr>
<tr>
<td><strong>FX11</strong></td>
<td>Specific LDHA inhibitor: a small-molecule inhibitor</td>
<td>In vitro / human lymphoma and pancreatic cancer xenografts</td>
<td>FX11 induced significant oxidative stress and cancer cell death.</td>
<td>Le et al. (2010)</td>
</tr>
<tr>
<td><strong>N-hydroxy-2-carboxy-substituted indole compounds</strong></td>
<td>Specific LDHA inhibitor: a small-molecule inhibitor</td>
<td>In vitro NMR experiments</td>
<td>Functional analysis of synthesized LDHA inhibitors</td>
<td>Granchi et al. (2011)</td>
</tr>
<tr>
<td><strong>Inhibition of LDHA by either RNA interference or pharmacological agents</strong></td>
<td>Inhibition of LDHA by either RNA interference or pharmacological agents</td>
<td>In vitro / in vivo; Several cancer cells.</td>
<td>Review of inhibition of LDHA by either RNA interference or pharmacological agents block tumor progress in vivo.</td>
<td>Oermann et al. (2012)</td>
</tr>
<tr>
<td><strong>Inhibition of LDHA by either RNA interference or pharmacological agents</strong></td>
<td>Inhibition of LDHA by either RNA interference or pharmacological agents</td>
<td>In vitro / in vivo; Cancers including breast cancer and hepatocellular carcinoma.</td>
<td>Review of inhibition of LDHA can block tumor growth, maintenance, and progression in vitro and in vivo.</td>
<td>Miao et al. (2013)</td>
</tr>
<tr>
<td><strong>shRNA mediated knockdown of LDHA</strong></td>
<td>Inhibition of LDHA by either RNA interference or pharmacological agents</td>
<td>In vitro; Breast cancer MDA-MB-435 cells</td>
<td>shRNA mediated knockdown of LDHA resulted in elevated mitochondrial ROS production and a concomitant decrease in cell proliferation and motility in breast cancer MDA-MB-435 cells.</td>
<td>Arseneault et al. (2013)</td>
</tr>
<tr>
<td>Inhibition of LDHA by either RNA interference*</td>
<td>Inhibition of LDHA by either RNA interference</td>
<td>Immunohistochemistry of human endometriosis samples; In vitro.</td>
<td>Hypoxia treatment induced the expression of LDHA; Silencing of LDHA expression displayed an impairment of mitochondrial function and promoted apoptosis while inhibited migration and glycolysis.</td>
<td>Zheng et al. (2021)</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>None</td>
<td>Schizophrenia</td>
<td>Experiments with schizophrenia brain</td>
<td>A significant increase in lactate in schizophrenia brain.</td>
<td>Pruett &amp; Meador-Woodruff (2020)</td>
</tr>
<tr>
<td>None</td>
<td>Autoimmune disease</td>
<td>Animal studies</td>
<td>Pro-inflammatory signals in autoimmune disease induced metabolic reprogramming, characterized by a shift to aerobic glycolysis.</td>
<td>Kornberg (2020)</td>
</tr>
</tbody>
</table>

*indicates results of preclinical studies on endometriosis.

SLC2A, Solute carrier family 2; GZFLC, Gui-Zhi-Fu-Ling-capsules; HK2, hexokinase 2; PBMCs, Peripheral blood mononuclear cells; PFKFB3, phosphofructokinase-2/fructose-2, 6-bisphosphatase 3; PKM2, pyruvate kinase isozyme; HSF1, heat shock factor 1; DCA, Dichloroacetate; IQ, Ilimaquinone; FX11, 3-dihydroxy-6-methyl-7-(phenylmethyl)-4-propynaphthalene-1-carboxylic acid;
Identification

Records identified through database searching

Screening

Records after duplicates removal

Records screened

Eligibility

Full-text articles assessed for eligibility

Removal of inappropriate articles by reading full-text articles

<table>
<thead>
<tr>
<th>Key words</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

| Identification | 117 | 364 | 5  | 16  | 17  | 3  | 5  |
| Records after duplicates removal | 26  | 246 | 1  | 1   | 2   | 0  | 1  |
| Records screened | 91  | 118 | 4  | 15  | 15  | 3  | 4  |
| Removal of inappropriate articles | 52  | 76  | 0  | 3   | 4   | 0  | 0  |
| Eligibility | 39  | 42  | 4  | 12  | 11  | 3  | 4  |
The Warburg Effect

- ROS
- Hypoxia
- TGF-β
- HIF-1
- LDHA
- Pyruvate
- LDHB
- Lactate
- Glucose
- PPP
- Glucose metabolism
- Glutamine
- Glutamate
- Fatty acids
- Acetyl-coA
- Redox homeostasis
- SOD

Mitochondria

- Malate
- Citrate
- α-KG
- Succinate
- Counterclockwise rotation of TCA cycle
- ATP
- ROS

Redox homeostasis

- ONOO⁻
- O₂⁻
- H₂O₂
- OH⁻