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The role of peripheral nerve signaling in endometriosis

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Abstract

A hallmark of endometriosis – a chronic debilitating condition whose causes are poorly understood – is neuronal innervation of lesions. Recent evidence demonstrates that the peripheral nervous system plays an important role in the pathophysiology of this disease. Sensory nerves, which surround and innervate endometriotic lesions, not only drive the chronic and debilitating pain associated with endometriosis but also contribute to a pro-growth phenotype by secreting neurotrophic factors and interacting with surrounding immune cells. The diverse array of contributions that neurons play in endometriosis indicate that it should be considered as a nerve-centric disease. This review is focused on the emerging field of exoneural biology and how it applies to the field of endometriosis, in particular the role that peripheral nerves play in driving and maintaining endometriotic lesions. A better understanding of the mechanisms of neuronal contribution to endometriosis, as well as their interactions with accompanying stromal and immune cells, will unearth novel disease-relevant pathways and targets, providing additional, more selective therapeutic horizons.

KEYWORDS

endometriosis, lesion, nerve signaling, nociceptor, peripheral nerves

1 | INTRODUCTION

Endometriosis is a common disorder affecting approximately 10% of reproductive age women that is defined by the presence of ectopic endometrial tissue growing in the peritoneal space. Ectopic lesions can grow throughout the peritoneal cavity but most commonly localize to fallopian tubes and ovaries as well as the tissue surrounding the pelvis.[1] Endometriosis is a highly variable disease which can present differently on a patient-by-patient basis. For some women, the primary symptoms of endometriosis might include dysmenorrhea and excessive bleeding during menstruation, other women may present with pain during intercourse, bowel movements, or urination, whereas other women will not know that they have been living with endometriosis until they seek treatment for infertility. Part of the symptom variability can be explained by lesion location, but the correlation between lesions size and symptomatology remains poorly understood.[2]

Over the last 20 years, our understanding of the mechanisms driving endometriosis lesion growth and pain presentation has evolved. Significant advances have been made in understanding how estrogen drives tissue pathology, resulting in aberrant inflammatory and neuronal states, and promoting invasion of lesions into the surrounding tissues.[3] Despite these advances, novel

treatments in endometriosis have lagged. Only one drug, ORILISSA (elagolix) has been approved for endometriosis in the past 20 years, a novel gonadotrophin-releasing hormone (GnRH) antagonist that targets the same pathway of hormone signaling as all previous endometriosis treatments.

In this review, we discuss the novel findings that endometriosis is a disease uniquely characterized by exoneural biology, whereby estrogen alters the neuronal biology to create and sustain a pathological tissue state that drives and sustains endometriotic lesion growth through multiple, interventional mechanisms. We start with an overview of patient symptoms and current treatment options, followed by an in-depth discussion on an emerging consensus in the literature that neurons can drive disease pathology and that estrogen is a key factor in this phenomenon. We end by discussing how this model identifies multiple non-hormonal treatments that could alter this pathological state and discuss how best to test the exoneural model of endometriosis that we lay out in this review.

2 | PATIENT PRESENTATION AND CURRENT TREATMENTS

It is difficult to describe the general characteristics of endometriosis patients for multiple reasons. First is the size and diversity of the patient population, as approximately 10% of all women will develop endometriosis during their lifetime.[4] Additionally, women with endometriosis often present with disparate symptoms (Figure 1) which can lead to very different paths to clinical diagnosis. Some women present young with pain-associated phenotypes such as dysmenorrhea, whereas other women have no pain symptoms and remain undiagnosed until later in life when they present with infertility. Summarizing the differences in these patient populations, Arruda et al., found that women whose chief complaints were infertility typically present older and have a much shorter time from symptom presentation to diagnosis with endometriosis (median 4 years), whereas women who present young with pain symptoms have a delay in over a decade before being diagnosed.[5] This multi-year delay in presentation to diagnosis is commonly observed across countries and studies.[5–7]

Another aspect driving heterogeneity of symptom presentation is the heterogeneity in location of the endometriotic lesions. Most common lesions are found in the peritoneal space, with a majority of patients presenting with lesions in the rectocervical region, 20%–40% with lesions on the ovaries, 12%–37% of patients presenting with lesions implanted along their gastrointestinal tract, and a minority of patients with lesions on the bladder or

ureters.[8] Part of the heterogeneity in pain presentation, for example, during bladder voiding, bowel movements, or during sex, is likely due to the heterogeneity in lesion location. Less commonly, patients can present with lesions on the fallopian tubes, leading to blockages of the fallopian tube, lesions on the appendix resulting in appendicitis-like presentations,[9] and in very rare cases patients can present with lesions in the thoracic cavity, in their limbs, or within the CNS all of which can present with unique symptomology.[10]

A final driver of heterogeneity in endometriosis presentation is in the histological subtype of lesions growing in each patient. Women with ovarian endometriosis often present with endometriomas or "Chocolate cysts" which contain old blood that is often dark brown colored and viscous. These lesions often impair fertility and can damage and degrade the healthy surrounding ovarian tissue. These women typically respond poorly to medical treatment, however surgical removal of the lesions is often fully effective in providing long-lasting relief when compared to surgical ablation of other forms of endometriosis.[11] Endometriosis can also present with a "gunshot" or "powder burn" morphology, which can contain fibrosis and scarring from damage to the surrounding tissue, and can be superficial or invasive in the host tissues such as with deep infiltrating endometriosis.[10]

After diagnosis, women with endometriosis have three classes of treatments available to them: (1) Analgesics to manage symptoms, (2) Hormonal therapies designed to inhibit estrogen-dependent growth of lesions, or (3) Surgical ablation of lesions. Beyond medical treatment, many women also find symptom relief during pregnancy or after menopause, although up to 2% of post-menopausal women continue to present with histologically confirmed endometriosis.[12]

As a disease largely characterized by pain, the most commonly used treatment for endometriosis are NSAID analgesics such as ibuprofen. While these drugs provide important pain-relief benefits to patients, they do not prevent further lesion growth or invasion of healthy tissue or address the infertility commonly associated with endometriosis. Additionally, the long-term use of NSAIDs such as ibuprofen or acetaminophen is increasingly associated with adverse health effects such as gastrointestinal and renal disorders. As women with endometriosis will likely experience symptoms for multiple days a month for decades, the side effects of long-term NSAID use are a serious consideration for this patient population.

The first line of treatment for women with endometriosis is treatment with oral contraceptives, with over 70% of women having used multiple forms of oral contraceptives to manage their symptoms.[13] It is important to note that use of these oral contraceptives is almost always an
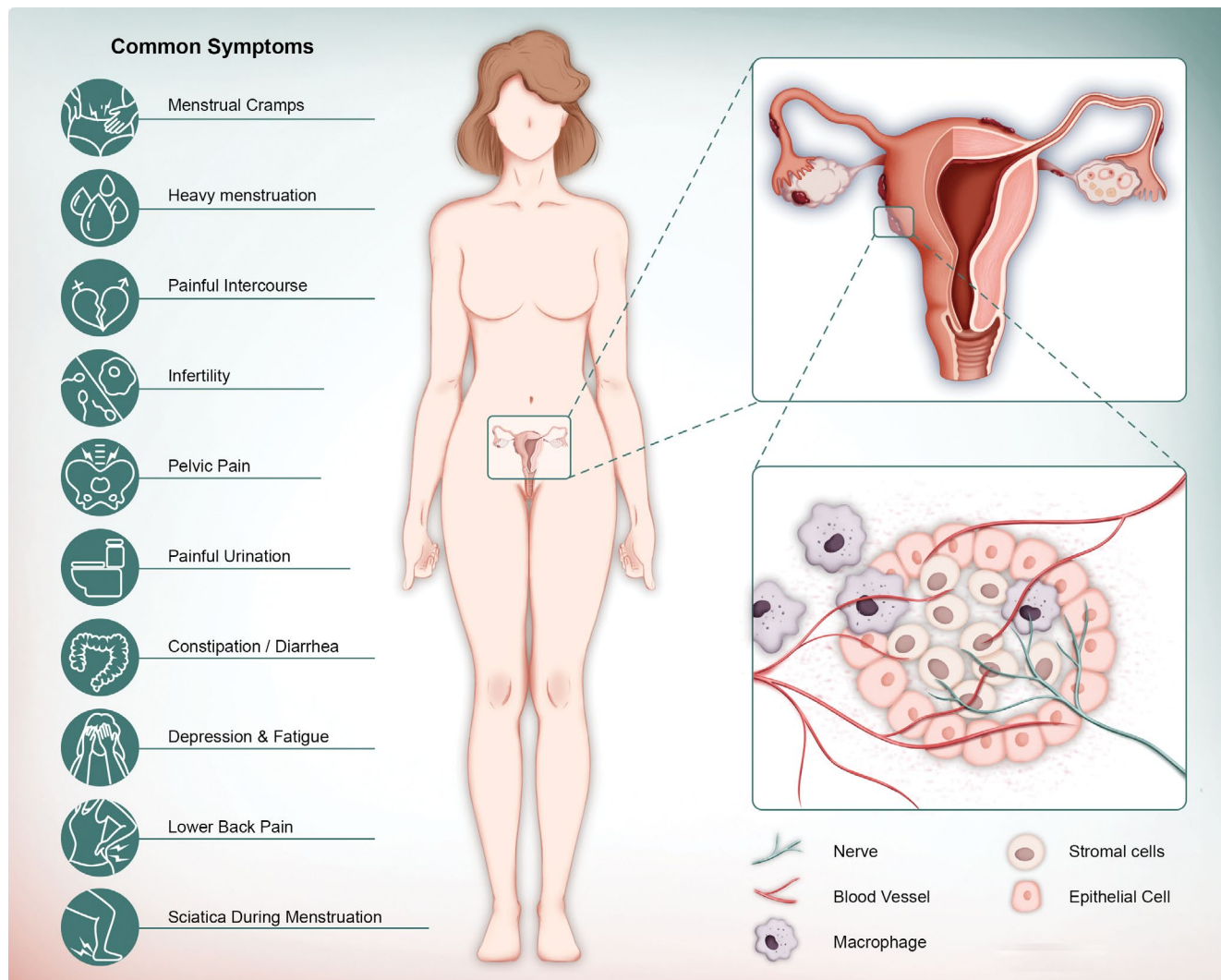
FIGURE 1 Overview of the broad and disparate symptoms associated with endometriosis. In endometriosis, lesion growth of endometrial tissue occurs outside of the endometrium. Lesions are composed of an abundance of distinct cell types including immune, stromal and epithelial cells as well as infiltrating blood vessels and nerves

"off-label" use of the medication as very few contraceptives have undergone clinical trials and approval for use in treating endometriosis.[14] Accordingly, there are relatively few clinical trials for any given oral contraceptive as to its effectiveness in disease management at any of the available doses of a drug. Due to this lack of clear clinical guidance, over 40% of women have tried between 3 and 10 different forms of oral contraceptive until they find a formulation that provides adequate symptom relief with an acceptable side effect profile.[13] Little research on side-by-side comparisons of the ability of any given contraceptive to provide symptomatic relief has been accomplished, forcing patients and clinicians to essentially find the right drugs through trial and error.

Approximately one third of endometriosis patients (~3% of all adult women prior to menopause) will fail to find an oral contraceptive that adequately manages their disease, likely due to the development of progesterone resistance.[13,15] These women have the option of taking aromatase inhibitors such as letrozole or anastrozole, which are also not FDA-approved for endometriosis,[14] but are generally able to confer prolonged periods of symptomatic relief in patients following surgical treatment.[16] For women, whose symptoms are not adequately managed by these drugs, third-line treatment options are available such as gonadotropin-release hormone agonists or antagonists. GnRH agonists and antagonists are clearly delineated from other endometriosis treatments as they are newer, have been studied in large-scale clinical trials, and FDA-approved for the treatment of endometriosis.[13]

As an alternative to oral contraceptives, surgical removal of endometriosis lesions can provide symptom relief and restore fertility in some women.[17] However, these procedures carry inherent risks of surgical complications

depending on lesion location and they are not effective in all women. Approximately half of women who initially experience symptom relief will eventually relapse and experience symptom and lesion return.[18,19]

While the current treatments for endometriosis are largely safe and effective, each treatment offers partial symptom relief at best and surgery remains the only effective approach in combating endometriosis-associated infertility. Thus, there is a clear need to understand the underlying biological mechanisms driving endometriosis growth and pain to design novel, fertility-sparing treatments that seek to cure endometriosis through lesion regression rather than focusing on symptom relief.

3 | EXONEURAL CHARACTERISTICS OF ENDOMETRIOSIS

3.1 | Elevated innervation is a hallmark of endometriosis

Significant work over the past 20 years has been performed to understand the mechanisms by which endometriotic lesions induce pain, the primary symptom of most patients. Much of this effort has focused on understanding the extent and type of nerves present in endometriotic lesions compared to surrounding tissues (Figure 2). While the relationship between lesions and pain will be discussed in detail in the ensuing sections, a wealth of literature has now found that endometriotic lesions are densely innervated.[20,21] In-depth analyses of the highly innervated lesions find that these nerves are largely sensory rather than sympathetic or parasympathetic in nature.[22,23] Interestingly, these analyses have also uncovered that endometriotic lesions are much more densely innervated than the surrounding peritoneal tissues that they invade,[20,24,25] suggesting that endometriotic lesions likely produce factors that drive sensory neurons to sprout neurites and innervate these lesions. The selectivity of this phenomena was shown in a 2013 report that found that peritoneal fluid from endometriosis patients directly stimulated neuritogenesis of purified dorsal root ganglion, but not sympathetic ganglion, in vitro.[26] Surprisingly, while peritoneal fluid from endometriotic patients supported sensory nerve growth, the peritoneal fluid from healthy women inhibited the growth of sensory neurons even when stimulated by exogenous nerve growth factor (NGF). Together, these data show that aberrant nerve growth is a key part of the pathophysiology of endometriosis. In this section, we review what is known about the neurotrophic and neuronal guidance factors produced by endometriotic lesions that could help explain this biology, and in subsequent sections we detail how this innervation

sustains lesion growth and likely underlies endometriosis-associated pain.

3.1.1 | Neurotrophins

The neurotrophins are a family of four proteins with known roles in supporting and directing neuritogenesis that become dysregulated and overexpressed in endometriotic lesions. The family includes NGF and its receptor TrkA, brain-derived neurotrophic factor (BDNF) and its receptor TrkB, neurotrophin-3 (NT-3) which binds to TrkC, and neurotrophin-4/5 (NT-4) which also binds to TrkB. Additionally, each of these neurotrophins can bind to pan-neurotrophin receptors such as p75/NGFR, SORT1, and ITGA9.[27–29]

3.1.2 | NGF/TrkA

NGF, the prototypical neurotrophic factor, has been well-studied in the context of endometriosis. The first group to evaluate NGF in endometriotic lesions found that it is broadly dysregulated in patients, with large increases in NGF expression in endometriotic lesions during the proliferative phase of a patient's estrus cycle[30] when estrogen levels are high. Consistent with this finding, estrogen strongly induces NGF production by macrophages in vitro,[31] and treatment of endometriosis patients with oral contraceptives drastically reduces the expression of NGF and one of its receptors, p75/NGFR.[32] In support of this model, oral contraceptive therapy reduces both NGF expression and nerve density in endometriotic lesions.[32]

3.1.3 | BDNF/TrkB

Like NGF, BDNF has been found to be dysregulated and overexpressed in ectopic but not in eutopic endometrial tissue.[33] Also, like NGF, BDNF's expression in macrophages is induced by estrogen[34] and seems to be abundantly expressed by the macrophages that infiltrate endometriotic lesions and by the stromal tissue surrounding these macrophages.[35] Interestingly, clues from endometrial cancer or the normal development of porcine endometrial tissue suggest that BDNF is a down-stream effector of estrogen signaling that mediates the pro-proliferative effects of estrogen.[36,37] Critically, the ability of estrogen to stimulate BDNF from macrophages seems to be mediated by the presence of neurons in the lesion, underscoring the critical cross talk between neurons and immune cells in endometriosis (Figure 2).[34]
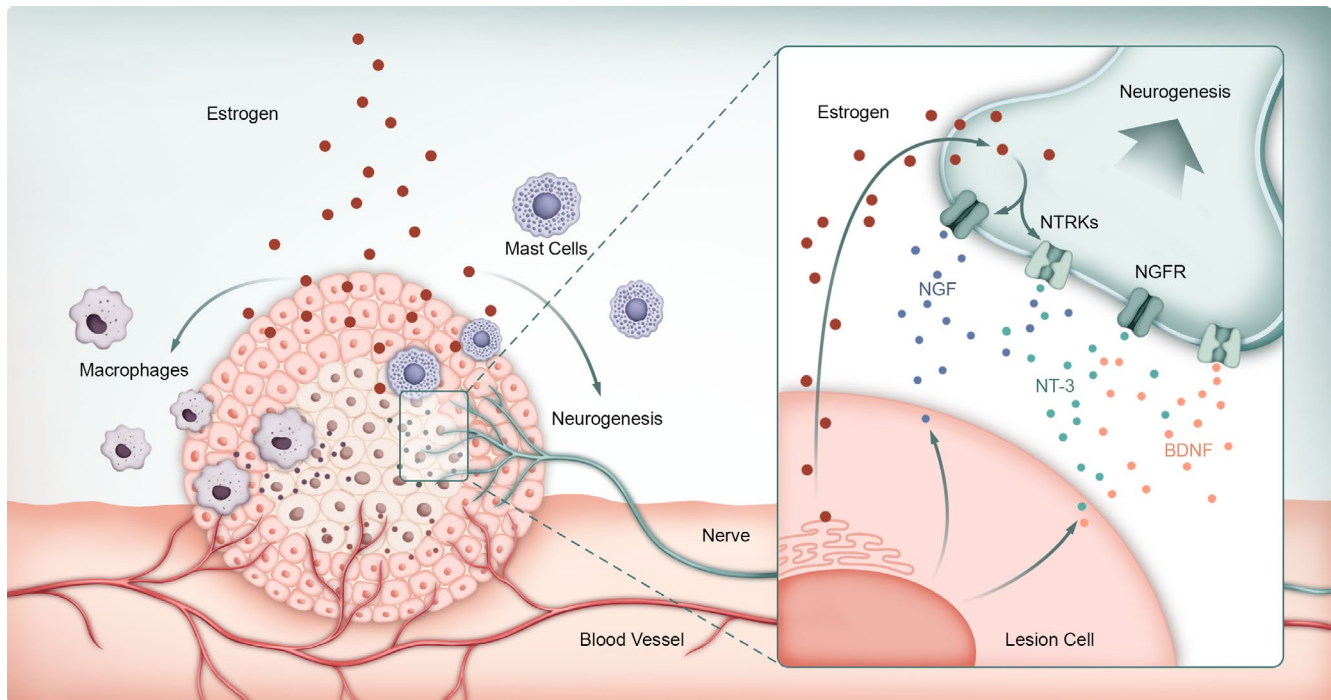
FIGURE 2 Multifaceted role of estrogen in endometriosis: Estrogen can mediate the recruitment of immune cells, nerve fibers, and blood vessels to lesions. Macrophages and mast cells once recruited, contribute to neurite outgrowth and peripheral nerve sensitization, respectively. Estrogen strongly induces neurotrophin production, including NGF, BDNF, and NT-3 by macrophages which signal through NTRK receptors on nerves to promote neurogenesis. Mast cell degranulation and the subsequent release of pro-inflammatory mediators can be triggered by estrogen release. Release of pro-inflammatory mediators from mast cells sensitizes peripheral nerve endings in endometriotic lesions, contributing to the pain. An estrogen-dependent detrimental cycle of macrophage-mediated neurogenesis and mast-cell mediated inflammation and sensitization drives the pro-growth cycle necessary for endometriosis progression. BDNF, Brain-Derived Neurotropic Factor; NGF, Nerve Growth Factor; NGFR, Nerve Growth Factor Receptor; NT-3, Neurotrophin-3; NTRK, Neurotrophic Tyrosine Receptor Kinase

3.1.4 | NT-3 and NT-4/5

As opposed to NGF and BDNF which have been extensively studied in endometriosis and found to be dysregulated and overexpressed, relatively few observations about NT-3 and NT-4/5 have been published to date. Both factors have been found to be expressed in the lesions,[38] but there is currently no data suggesting that they play a critical role in endometriosis pathophysiology.

3.1.5 | Neuronal guidance factors

In addition to neurotrophins, endometriotic lesions express high levels of multiple neuronal guidance factors which can attract or repel neurite growth into a tissue. These neuronal guidance factors include the semaphorins, and their receptors neuropilin-1 and neuropilin-2, plexins, as well as other secreted factors and receptors. In normal human endometrial tissue, expression of many semaphorins is upregulated in the proliferative stage of the menstrual cycle, when estrogen is at its highest.[39]

Estrogen has been found to induce the expression of semaphorins, such as SEMA3F, in uterine tissue.[40] The semaphorins are well characterized as neuronal repelling factors which function by binding to neurons expressing their cognate receptors and preventing innervation of tissues. In the peritoneum of endometriosis patients, noradrenergic sympathetic neurons express these receptors,[40,41] and compellingly sympathetic innervation of endometriotic lesions tends to be very low, especially when compared to the highly abundant sensory innervation seen in endometriosis.[41,42]

The complex interplay between neuronal repulsive and attractive guidance factors is crucial to the innervation of endometriotic lesions. Alongside neurogenesis of lesions, intricate vascularization often occurs in parallel. Evidence suggests that endometriotic lesions develop in areas of rich vascularization and that angiogenesis is a key contributing factor in lesion growth and maintenance.[43] Focus has shifted in the field to the role of neuroangiogenesis, the coordinated infiltration of both nerves and blood vessels into lesions, and to the identification of common molecular mechanisms underlying this highly regulated

process.[44] In the context of endometriosis, semaphorin 3C was found to be upregulated in gene expression microarrays in the endometrium of women with endometriosis.[44] Semaphorins, as mentioned above are neuronal repelling factors, but also have strong affinity for the vascular endothelial growth factor (VEGF) and are known to play a role in angiogenesis.[45]

3.2 | Nociceptor signaling and dysregulation in endometriosis

Pain is the primary symptom of endometriosis and is the most common reason for seeking treatment. Patient reports describe the pain associated with endometriosis as negatively impacting upon daily life[46] and estimates suggest that up to $22 billion is lost annually in health care costs and reduced productivity as a result.[47,48] Dysmenorrhea is the most common pain associated with endometriosis[49] while others include dyspareunia,[50] dyschezia, and chronic pelvic pain.[51] Pain may be nociceptive, inflammatory, or neuropathic in nature, all three of which may be present in endometriosis at various stages of disease progression.

Endometriosis is defined as the presence of endometrium in abnormal or ectopic locations. These ectopic pieces of endometrial tissue are known as lesions and are most commonly found in the pelvic area, including ovarian and peritoneal regions.[52] A strong body of evidence demonstrates the presence of nerve fibers in endometriotic lesions.[20,24,25] Early work in this field demonstrated the presence of substance P (SP), calcitonin gene-related peptide (CGRP), acetylcholine (Ach), or tyrosine hydroxylase (TH) positive[24] fibers in endometriotic lesions. Moreover, many of these fibers were co-localized and the overall density of nerve fibers was greater in patients with peritoneal endometriotic lesions compared to women with normal peritoneum. Findings from the same group showed a similar trend in ovarian endometriotic lesions.[24] It was originally postulated that there was a relationship between the presence and/or number of lesions and the degree of pain reported by patients. Other theories propose a spatial relationship between lesions and nerves fibers, suggesting that such proximity is indicative of increased pain.[53] Conversely clinical studies show that many lesions are not associated with nerve fibers, yet patients still report pain.[54,55]

The presence of nociceptors in lesions suggests a role of these nerves in driving pain associated with endometriosis.[56] Multiple lines of evidence suggests that pelvic and visceral small diameter nociceptors are estrogen sensitive.[57,58] Activation of the estrogen receptor ERα in sensory nerves enhanced bradykinin-induced thermal allodynia in female rodents.[59] It is clear that estrogen plays a prominent role in the pathophysiology of endometriosis by mediating the interaction between macrophages and nerves, as well as regulating neurogenesis.[60] Estrogen-dependent release of the pro-inflammatory cytokine IL-6 from peritoneal macrophages is documented in women with endometriosis[61] as well the observation of increased NGF release from estrogen-dependent mast cell degranulation.[62] However, the estrogenic modulation of pain is known to be highly complex and poorly understood, with estrogens reported to have pro- and anti-nociceptive effects, often depending on the type of pain in question. Further work is clearly needed to better understand the relationship between endometriosis pain.

Estrogen is also a well-known modulator of the Wnt signaling pathway, which is commonly upregulated in endometriotic tissues.[63] Wnt signaling, via its canonical β-catenin pathway, regulates essential nervous system processes such as axonal guidance, synapse formation, and neuronal migration and polarization.[64–66] In the context of endometriosis, dysregulated activation of the Wnt/β-catenin signaling pathway contributes to the fibrous tissue commonly found in endometriotic lesions,[67] as well as the invasive and metastatic phenotype of endometriotic lesion cells.[68] Interestingly both Wnt3a and β-catenin are upregulated in the dorsal horn of the spinal cord in mice in various different pain models including inflammatory and neuropathic models.[69] As the pain in endometriosis is considered to be inflammatory and neuropathic in nature, interest is growing in the viability of the Wnt/β-catenin signaling pathway as a potential therapeutic target in endometriosis.[70] The highly interconnected nature of neurons, estrogen, and Wnt/β-catenin pathway provides evidence for an important role of peripheral nerves in endometriotic tissues and further strengths the hypothesis that endometriosis is a disease characterized by exoneural biology.

3.2.1 | Role of ligand-gated ion channels in endometriosis-associated pain

Transient receptor potential vanilloid 1 (TRPV1), a nonselective cation channel that is sensitive to heat and capsaicin is expressed in peritoneal endometriotic lesions.[71] Furthermore, TRPV1 was more prevalent in patients with endometriosis, although no correlation with pain intensity, duration, or stage of disease progression was found.[71] In rodent models of endometriosis, elevated TRPV1 expression was observed in dorsal root ganglia (DRG) and peritoneal lesions.[72,73] DRG's from mice with endometriosis were more responsive to capsaicin treatment compared to sham animals, providing further evidence for elevated TRPV1 expression in this condition.[73]

In addition to TRPV1, voltage-gated sodium channels which control the firing of nociceptors and whose role in pain are well documented,[74] have been hypothesized to play a role in endometriosis-associated pain.[71] Gene expression of SNC9A and SCN11A which code for voltage-gated sodium channels, Nav1.7 and Nav1.9, respectively, were found to be elevated in endometriotic lesions of women.[75] Expression of sodium channels in sensory nerves, such as those that innervate endometriotic lesions, appear to be estrogen dependent as incubation with estrogen agonists upregulated SCN9A and SCN11A expression.[75]

Purinergic receptors are ligand-gated ion channels that are expressed in sensory neurons which can be activated by adenosine triphosphate (ATP). P2X3 is one such purinergic receptor whose expression in the endometrium of women with endometriosis was significantly higher compared to control endometrium, and interestingly was also correlated with the severity of pain reported.[76] Estrogen appears to modulate the response of P2X3 receptors to ATP at the level of the DRG, which is a key convergence point for visceral afferents that promote pelvic pain.[77] Current interest in targeting P2X3 in endometriosis is high with multiple pharmaceutical companies having initiated drug programs, one of which has begun to enter Phase II clinical trials (NCT03654326).

Aside from the nociceptive-specific ion channels described above, several other ion channels are postulated to play a role in the pathogenesis of endometriosis, including aquaporins, chloride channels, and cystic fibrosis conductance regulator.[78] Further work in this area is warranted but careful consideration should be taken when accounting for the underwhelming clinical data of ion channel blockers (particularly sodium channels) for the treatment of other chronic pain conditions.[79,80]

3.3 | Neuro-immune interactions in endometriotic lesions: Peripheral sensitization

The wide variety of sensory nerve fibers present in endometriotic lesions, may help to explain the chronic cyclical nature of pain associated with this condition. Accompanying these nerve fibers are different types of immune cells and the interaction of these can cause the release of various pro-inflammatory mediators. The release of these pro-nociceptive and pro-inflammatory mediators (termed neurogenic inflammation) can sensitize the surrounding sensory nerve fibers and is responsible for driving many chronic pain conditions via a process known as peripheral sensitization.[81] The various types of immune cells that contribute to peripheral sensitization in endometriotic lesions are discussed below.
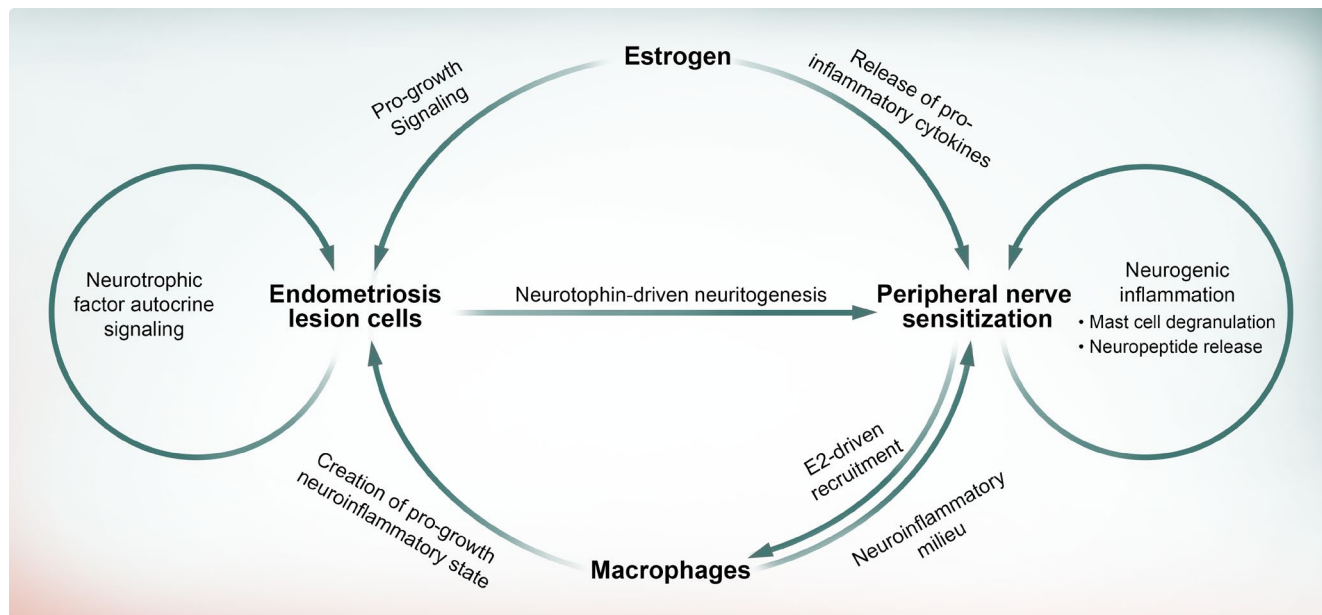
3.3.1 | Macrophages

Macrophages are extremely abundant cells in endometriotic lesions in both patient samples and rodent models of endometriosis.[82] The macrophages found in endometriosis patients tend to display a more activated state[83] and are found to promote disease progression.[82,84] Consistent with an altered inflammatory milieu, patients with endometriosis are documented to have elevated levels of multiple cytokines in their peritoneal fluid.[85] Critically, macrophages in endometriotic lesions promote the high levels of sensory innervation and sensitization seen in endometriosis[34,86] which can drive both lesion growth and the pain commonly associated with endometriosis, as described above. In vitro, macrophages have been found to be recruited by sensory neurons in an estrogen-dependent fashion, and to subsequently promote neurite outgrowth of sensory neurons through neurotrophins like BDNF, NT-3, or other factors such as IGF-1.[34,86] Furthermore, macrophages can support the growth of the lesion itself, leading to further neurite outgrowth as well as damaging the surrounding tissue, further enhancing the inflammation. Combined, these signals create a self-propagating loop of enhanced innervation, inflammation, and lesion growth. This self-propagating relationship between nerves, macrophages, and estrogen is reviewed in depth in Liang et al., 2018.[3]

More recent work has found that endometriosis lesions in rodent models of the disease are derived from both eutopic endometrium-resident macrophages, peritoneal-resident macrophages, as well as monocyte-derived macrophages.[87] Understanding the specific contribution of each sub-population of macrophages to the growth of endometriosis lesions as described above (Figure 3) is an ongoing effort in the field. In one set of experiments, depletion of macrophages from donor mice prior to implantation led to decreased lesion growth, whereas depletion of macrophages from recipient mice led to enhanced lesion growth,[87] suggesting peritoneal and blood-derived monocytes largely act to suppress lesion growth. Conversely, another recent study using endometriotic lesion engraftment into a mouse model enabling specific ablation of CD206+ M2 macrophages found that loss of these M2 macrophages strongly reduced lesion growth.[88] More work is needed to reconcile these findings and to better elucidate the roles of different macrophage subtypes more clearly in endometriosis lesion growth.

3.3.2 | Mast cells

Mast cells are potent mediators of IgE-mediated allergic responses and have the ability to respond rapidly to innate IgE-dependent stimuli.[89] Mast cells are implicated in

FIGURE 3 Schematic proposal for the inter-dependent nature of estrogen, macrophages, and peripheral nerve fibers in driving endometriotic lesion growth. Peripheral nerves not only drive sensitization and pain associated with endometriosis but can also promote recruitment of macrophages to lesions, resulting in a pro-growth inflammatory environment

the pathophysiology of several different diseases including rheumatoid arthritis,[90] migraine[91] multiple sclerosis[92] as well as endometriosis[93] (Figure 2). Indeed, a large body of clinical and pre-clinical evidence support a role of mast cells in the pathophysiology of endometriosis.[89,94,95] In patients diagnosed with endometriosis, infiltration of mast cells was consistently observed across the stroma of lesion areas.[96] Furthermore, the number of degranulated or activated mast cells was significantly higher in endometriosis patients compared to controls. Upon degranulation mast cells release a host of vasoactive, pro-inflammatory, and pro-algesic mediators (e.g., histamine, TNF-α, NGF, and IL-6), capable of inducing and sustaining a local inflammatory environment. Mast cell degranulation can directly activate and sensitize sensory nerves contributing to the development of hyperalgesia[97,98] although this has yet to be demonstrated in the context of endometriosis.

Targeting sensory nerve and mast cell interactions is hypothesized to be an effective approach in combating pain.[99] One target that may be of interest is the chemokine CCL8, which was upregulated in mast cells upon co-culture with endometrial cells and also increased the proliferation and migration of endometrial epithelial cells.[100] Expression of CCL8 and its receptor CCR1 were overexpressed in the ectopic endometrium, where it was co-localized with blood vessels, a finding which confirms earlier reports of high concentrations of mast cells around blood vessels in lesions[101] and inhibition of CCR1 attenuated angiogenesis in a mouse model of endometriosis.[100]

Additional mast cell targets that may be of interest in treating endometriosis include Janus Kinase 3 (JAK3)

which is highly expressed in both murine and human mast cell and plays a key role in IgE-mediated cell degranulation.[102] Indeed, JAK3 inhibitors have shown early promise in several pre-clinical inflammatory models including peritonitis, colitis, and airway inflammation.[103]

4 | THERAPEUTIC HORIZONS: EXONEURAL TREATMENTS FOR ENDOMETRIOSIS

Given the essential role of nerves in driving disease progression and in particular sensory neurons in driving the pain phenotype that characterizes endometriosis (Figure 3), there is a clear need for targeted research to better understand the mechanisms by which neurons contribute to this progressive disease. It is our belief that a greater understanding of the critical role that neurons play in endometriosis progression will enable the discovery of a new class of targets for the treatment of endometriosis. Importantly, these exoneural targets are likely to be independent of estrogen signaling which is a key criterion for developing fertility-preserving treatments.

An example of an exoneural approach to treating endometriosis is seen in recent work on the role of the vagus nerve in regulating disease pathology. Surgical denervation of the vagus nerve (vagotomy) in mice can accelerate the development and growth of lesions in a mouse model of endometriosis, as well as significantly reducing the thermal pain associated with endometriosis.[104] Interestingly, the same study also demonstrated a reduced

vagal tone in women with ovarian endometriosis. To determine the contribution of vagal nerve signaling further in endometriosis, the authors also stimulated the vagal nerve in mice resulting in a reduction of lesion growth and development.[105] The above work provides compelling evidence of a role for vagal nerve dysfunction in driving endometriosis which could lead to novel treatment options and highlights the importance of understanding the importance of neural-disease interactions.

By applying other denervation approaches to the available surgical and non-surgical in vivo models of endometriosis,[73,106,107] key translational disease characteristics such as alterations in pain behavior, lesion size, or lesion number can be evaluated. Alternatively, as nerve denervation/ablation is an irreversible procedure and nerve damage is known to promote a cytotoxic environment surrounding the injured nerve,[108] approaches that transiently silence or activate peripheral nerves may be more beneficial in the context of endometriosis. Multiple models of sensory neuron ablation currently exist ranging from surgical[109] to chemical[110] to more recent and more selective transgenic mouse approaches.[106] For chemical ablation approaches, both resiniferotoxin and 6-hydroxydopamine (6-OHDA), which target sensory and sympathetic nerves, respectively, are well characterized methods of nerve ablation and could be readily applied to numerous pre-clinical in vivo endometriosis models.

By evaluating endometriosis as an exoneural disease, commonalities between endometriosis and other diseases, such as cancer, emerge. For example, enhanced innervation of both endometriotic lesions and cancers has been observed, and in some cases this neurite outgrowth has been attributed to common factors such as NGF.[32,111] Recent studies have even described high levels of perineural invasion in endometriosis, a common feature of invasive cancers,[3,112] although the incidence in endometriosis remains to be established through further studies. In cancer, the role of neurons in promoting angiogenesis ("neuroangiogenesis") is well established, and work in endometriosis suggests a similar mechanism may drive vascularization.[43,44] Furthermore, cancer-associated pain, which can often be nociceptive or neuropathic in nature, similar to endometriosis pain, is related to the degree of peripheral nerve innervation of specific tumors.[113,114] By applying the lessons from cancer and other disease indications to endometriosis, it is possible that novel therapeutic targets may become visible for endometriosis.

5 | CONCLUSIONS

The principles of exoneural biology are readily applicable to the field of endometriosis and doing so will help elucidate the precise contributions of peripheral nerves to this perplexing and under-served disease. Establishing new disease-modifying therapies for endometriosis remains a high priority. The heretofore underappreciated role of peripheral nerves is ripe of exploitation to deliver tractable, long-lasting treatments which pave the way for a brighter future in combating this debilitating disease.

CONFLICT OF INTEREST
Godin SK, Wagner J, Huang P, and Bree D, are employees of Cygnal Therapeutics.

AUTHOR CONTRIBUTIONS
Godin SK and Bree D contributed equally to the concept, design, and writing of the review. Wagner J and Huang P assisted in the concept and revision of the review.

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