1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

Nerve Fibers and Endometriotic Lesions:

Partners in Crime in Inflicting Pains in Women with Endometriosis

Dingmin Yan¹, M.D., Xishi Liu^{1,2}, M.D., Ph.D., Sun-Wei Guo^{1,2}, Ph.D.

¹ Shanghai OB/GYN Hospital, Fudan University, Shanghai 200011, China

² Shanghai Key Laboratory of Female Reproductive Endocrine-Related Diseases,
Fudan University, Shanghai, China

Grant support: This research was supported in part by grants 81270676 (SWG),
81471434 (SWG), 81530040 (SWG), 81070470 (XSL), and 81370695 (XSL) from
the National Science Foundation of China, and grant 2013ZYJB0019 (XSL) from
Shanghai Municipal Commission of Health and Family Planning.

Running title: Nerve fibers and endometriotic lesions

Conflict of interest statement: None declared.

Address correspondence and reprint requests to: Sun-Wei Guo, Ph.D. Shanghai
Obstetrics and Gynecology Hospital, Fudan University, 419 Fangxie Road,
Shanghai 200011, China (FAX: 86-21-6345-5090; Email: hoxa10@outlook.com)

Abstract

One of major objectives in treating endometriosis is to alleviate pain since dysmenorrhea and other types of pain top the list of complaints from women with endometriosis who seek medical attention. Indeed, endometriosis-associated pain (EAP) is the most debilitating of the disease that negatively impacts on the quality of life in affected women, contributing significantly to the burden of disease and adding to the substantial personal and societal costs. Unfortunately, the mechanisms underlying the EAP are still poorly understood. In the last two decades, one active research field in endometriosis is the investigation on the distribution and genesis of nerve fibers in eutopic and ectopic endometrium, and the attempt to use endometrial nerve fiber density for diagnostic purpose. Since EAP presumably starts with the terminal sensory nerves, in or around endometriotic lesions, that transduce noxious mediators to the central nervous system (CNS) which ultimately perceives pain, this field of research holds the promise to elucidate the molecular mechanisms underlying the EAP, thus opening new avenues for novel diagnostics and therapeutics. In this review, we shall first briefly provide some basic facts on nerve fibers, and then provide an overview of some major findings in this field while also note some conflicting results and expose areas in need of further research. We point out that since recently accumulated evidence suggests that endometriotic lesions are wounds undergoing repeated tissue injury and repair, the relationship between endometriotic lesions and nerve fibers is not simply unidirectional, i.e. lesions promote hyperinnervations. Rather, it is bidirectional, i.e. endometriotic lesions and nerve

1 43 fibers engage active cross-talks, resulting in the development of endometriosis and
2
3 44 pain. That is, nerve fibers and endometriotic lesions are actually partners in crime in
4
5
6 45 inflicting pains in women with endometriosis, aided and abetted possibly by other
7
8
9 46 culprits, some yet to be identified. We provide a list of possible perpetrators likely to
10
11
12 47 be involved in this crime. Finally, we discuss possible implications when viewing the
13
14
15 48 relationship from this vista.
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

50 **Keywords:** Cross-talk; endometriosis; nerve fibers; pain

53

54

55

68

75 processing regions [16, 17], and may also have sustained activation of descending
76 pain facilitatory pathways from the brainstem [18], so far much effect in EAP
77 research is focused on the identification of nociceptors in general and nerve fibers in
78 particular.

79
80 In this review, we shall first briefly provide some basic facts on nerve fibers, and then
81 provide an overview of some major findings in this field while also note some
82 conflicting results and expose areas in need of further research. We point out that
83 since accumulating evidence suggests that endometriotic lesions are wounds
84 undergoing repeated tissue injury and repair (ReTAIR) [19-21], the relationship
85 between endometriotic lesions and nerve fibers is not simply unidirectional, i.e.
86 endometriotic lesions simply just promote innervations. Rather, it is bidirectional, i.e.
87 endometriotic lesions and nerve fibers engage active cross-talks, resulting in the
88 development of endometriosis and pain. That is, nerve fibers and endometriotic
89 lesions are actually partners in crime in inflicting pains in women with endometriosis,
90 aided and abetted possibly by other culprits, some yet to be identified. We provide a
91 list of possible perpetrators likely to be involved in this crime. Finally, we discuss
92 some possible implications when viewing the relationship from this vista. Due to
93 space limitations, we shall not delve into possible pain pathways involved in EAP.

94

95 **A primer on nerve fibers**

96

1 97 The perception of pain is mediated by a crosstalk between the CNS and the peripheral
2
3 98 nervous system (PNS) that is exerted by afferent and efferent nerves. Sensory nerves
4
5
6 99 belong to afferent nerves that carry nerve impulses from peripheral receptors toward
7
8
9 100 the CNS. Based on their sizes, peripheral nerve fibers can be classified as three types
10
11
12 101 [22] (Table 1). These sensory nerves include chemo-, mechano-, photo-, and
13
14
15 102 thermo-receptors, as well as nociceptors. Thousands of afferent neuronal cell bodies
16
17
18 103 are aggregated in a swelling just outside the spinal cord known as the dorsal root
19
20 104 ganglion (DRG).

21
22
23 105
24
25

26
27 106 **(Table 1 and Figure 1 about here)**
28
29

30 107
31
32

33
34 108 The efferent nervous system includes the motor nerves and the autonomic nerves
35
36 109 involved in the function of internal organs. The autonomic nervous system (ANS) is
37
38
39 110 comprised of two branches, the sympathetic and the parasympathetic nerves.
40
41
42 111 Depending on the neurotransmitters released from the axon terminals, the efferent
43
44
45 112 nervous system can be further divided into cholinergic and adrenergic nerves. The
46
47
48 113 former, which include all preganglionic fibers and parasympathetic postganglionic
49
50
51 114 fibers, release acetylcholine (ACh) while the latter, which include almost all of
52
53
54 115 sympathetic postganglionic fibers, are specialized to release norepinephrine (NE) [23,
55
56 116 24] (Figure 1).

57
58
59 117
60
61

Neural density and neural remodeling in endometriosis and adenomyosis

119

The earlier attempts in the late 1990s to find the anomaly, if any, in nerve fibers in endometriosis were unfruitful [25, 26]. In fact, an earlier study found no nerve fiber in endometriomas [27]. However, Anaf's group was the first to report possible involvement of nerve fibers in the EAP [28]. Based on the observation that uteri from patients with chronic pelvic pain showed proliferation of small-diameter nerve fibers throughout the myometrium [29], Quinn postulated that nerve injury (denervation) in uterus and/or uterosacral ligaments, due to either difficult intrapartum episodes in parous women or persistent straining to achieve defecation in nulliparous women, followed by re-innervation in the uterine isthmus may be the primary source of many clinical symptoms, and retrograde menstruation with adhesion of endometrium to injured tissue surfaces may lead to endometriosis [30-32].

Firm evidence for hyperinnervation in endometriotic lesions came from animal experimentation performed by neurobiologist Berkley's group [33, 34], which was soon confirmed in human endometriosis [35]. The interest in nerve fibers was catapulted to a new height when Fraser and his associates published a series of studies showing the presence of nerve fibers in endometrium from women with endometriosis but the complete absence in women without, and reported an unprecedented 100% sensitivity and 100% specificity in classifying patients based on the presence of nerve fibers in endometrium [36-42]. Challenges in differential diagnosis aside, the rosy

prospect of a novel, minimally invasive diagnostic biomarker with perfect sensitivity and specificity generated a great deal of excitement, prompting many investigations in this field.

Given the nature of chronic, visceral pain in endometriosis, the great majority of endometrial nerve fibers are reported to be unmyelinated C fibers, while myelinated A δ fibers may also be involved [43]. An array of specific neural markers, such as protein gene product 9.5 (PGP9.5), substance P (SP), calcitonin gene-related peptide (CGRP), and tyrosine hydroxylase (TH) have been reported to be stained positive in nerve fibers in or surround endometriotic lesions of different types, and the consensus is that there is hyperinnervation in both ectopic and eutopic endometrium from women with endometriosis [28, 34, 35, 38, 39, 44-63] (Table 2). Compared with other pelvic lesions, deep infiltrating endometriosis (DIE) seems to present a higher density of nerve fibers [64]. Adenomyosis, once considered to be endometriosis *interna*, appears to be different from endometriosis, in that both the functional layers and myometrium of women with adenomyosis had a tendency to show fewer nerve fibers compared with women without [44, 52, 65].

(Tables 2 and 3 about here)

While the neural density in lesions has been reported to be positively associated with

1 161 the severity of pelvic pain as compared with painless gynecologic conditions [28, 45,
2
3 162 50], other gynecological conditions with pain are also reported to be hyperinnervated
4
5
6 163 in endometrium, irrespective the pain is caused by adenomyosis, uterine fibroids, or
7
8
9 164 peritoneal adhesions [48, 49, 54, 66, 67]. This seems to be consistent with the report
10
11
12 165 that while endometriosis accounts for nearly one-third of cases with chronic pelvic
13
14
15 166 pain, the majority of cases are due to conditions other than endometriosis, such as
16
17
18 167 adhesions and infections [68]. These results, taken together, raise the issue as whether
19
20
21 168 the nerve fiber density in endometrium can be used as a minimally invasive diagnostic
22
23
24 169 procedure, especially given the report that eutopic endometrium from women with
25
26
27 170 endometriosis does not contain neurotrophic factors [55].
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

171
172 Table 3 summarizes the results of using endometrial nerve fiber density as a potential
173 diagnostic tool for endometriosis. It can be seen from Table 3 that endometrial nerve
174 fibers are present, but not exclusively, in women with endometriosis. In fact, they may
175 only be associated with the presence of pain symptoms irrespective pathological
176 conditions. One recent Cochrane review concludes that, despite “promising evidence
177 of diagnostic accuracy”, “there was insufficient or poor quality evidence for any
178 clinical recommendations” based on endometrial nerve fiber density [69]. Aside from
179 the evidence for increased neural density in endometriosis, there is also evidence for
180 neural remodeling. A recent study showed decreased sympathetic nerve fibers in the
181 mucosal and muscular layer near the intestinal endometriosis [60], and this is
182 confirmed by the demonstration of an imbalance in innervation between nerve fibers

in peritoneal lesions, with an increase of sensory nerves and a decrease of sympathetic nerves [61]. The latter finding appears to be at odds with the finding from the rat model of endometriosis, in which the ectopic lesions induce not only sensory but also autonomic innervations [34]. Furthermore, in comparison to healthy women, the density of sympathetic nerve fibers in the non-lesional, unaffected peritoneum of women with endometriosis is significantly reduced, while that of sensory nerve fibers remains the same [70]. A recent study demonstrated higher expression of Semaphorin 3A, a neuronal chemorepellent acting as an important axonal guidance molecule [71], in peritoneal endometriosis and deep endometriosis of uterosacral ligament, paralleled with reduced density of sympathetic nerve fibers [72]. This seems to suggest that endometriotic lesions may secrete molecules that regulate the neural remodeling.

It should be noted that the extensive research in nerve fibers in endometriosis, published so far, still revealed little of the mechanisms underlying pain perception in EAP. It has been postulated that in situ menstruation within endometriotic lesions elicits a sterile, low-grade inflammatory response in peritoneal cavity causing the pelvic pain. The proposed pro-inflammatory and neuro-angiogenic milieu, manifested by increased recruitment and accumulation of activated macrophages and mast cells with increased production (cytokines, growth factors, angiogenic factors) as potent mediators of neuro-inflammation, contributes to neurogenesis in endometriosis [73].

Neurotrophic factors and pain mediators secreted by endometriotic lesions

204

205 Despite the seemingly dashed hope in the use of endometrial nerve fibers as a
206 diagnostic for endometriosis, the interest in nerve fibers has not been abated since
207 hyperinnervation in endometriosis is now fully established. Consequently, a question
208 arises as what causes the hyperinnervation, or the increased nerve fibers. This
209 prompted research on neurotrophic factors (NTFs) secreted by endometriotic lesions
210 that drive the hyperinnervation.

211

212 Mammalian NTFs refer to a family of peptides mediating neuronal growth, survival
213 and differentiation, which contains nerve growth factor (NGF), brain-derived
214 neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) and NT-4/5 [74]. Different
215 NTFs have varying binding specificity for different receptors. For example, NGF
216 binds preferentially to the high-affinity receptor tyrosine receptor kinase A (TrkA),
217 BDNF and NT-4 to TrkB, and NT-3 to TrkC. In association with each of these Trks,
218 the NGF low-affinity receptor p75 can increase the their affinity for their
219 corresponding NTFs [75].

220

221 Endometriotic lesions express both TrkA and p75 [35, 63, 65]. NGF, but not TrkA,
222 was found in endometrial luminal epithelium of healthy women, suggesting that NGF
223 selectively promotes neurite outgrowth from a subset of NGF-responsive neurons.
224 Blocking of p75 attenuates NGF-induced excitability of nociceptors in culture [76],

1 225 raising the possibility that p75 might also have a role in pain. Given that both TrkA
2
3 226 and p75 staining was significantly reduced after hormonal treatment, estrogen could
4
5
6 227 be a factor in the upregulation of NGF and its receptors [40, 41, 77, 78].
7
8
9

10 228
11
12
13 229 NTFs have been shown to play significant roles in the mediation of neural sprouting
14
15
16 230 and hypertrophy, which, in turn, may be responsible for increased neural density in
17
18
19 231 endometriosis and EAP [79, 80]. The peritoneal fluid (PF) from women with
20
21
22 232 endometriosis is shown to contain NTFs such as NGF that promote neurite outgrowth,
23
24 233 and NGF blockade significantly reduces the outgrowth [81]. It also contains NT-3, but
25
26
27 234 not BDNF [82]. However, endometriotic lesions express BDNF and NT-4/5 [83].
28
29
30 235 Plasma BDNF, but not NGF or NT-4/5, levels are also reported to be higher in women
31
32
33 236 with endometriosis [84]. Consistent with the neurotrophic propensity of endometriotic
34
35
36 237 lesions, growth-associated protein 43 (GAP43), a marker for neuronal sprouting and
37
38
39 238 neurite outgrowth [85], has been consistently identified in lesions in humans [62, 63,
40
41 239 86] as well as in animal models [87].
42
43
44 240

45
46
47 241 Aside from these traditional NTFs, other molecules may also act as NTF. For example,
48
49
50 242 thromboxane A2 (TXA₂) is reported to stimulate neurite outgrowth in cortical neurons
51
52
53 243 [88]. This is important since endometriotic stromal cells secrete TXA₂ [89], and we
54
55
56 244 recently found that it acts as an NTF in DRG-derived neurons (Yan et al., submitted
57
58
59 245 for publication).
60
61
62
63
64
65

246

247 As an NTF, NGF also lowers the threshold of certain nociceptors, and stimulates the
248 release of pain-associated neuropeptides from small sensory neurons, leading to
249 hyperalgesia [90]. Transient receptor potential vanilloid 1 (TRPV1), an integrator of
250 multiple pain stimuli that has been reported to be over-expressed in endometriotic and
251 adenomyotic lesions accompanied with dysmenorrhea or pelvic pain [91-93], may
252 also be involved in nociceptor sensitization through its potentiation [94]. Mediated by
253 TrkA activation, NGF could increase TRPV1 expression. Estrogen may also cause
254 TRPV1 over-expression in sensory neurons [95]. Thus, NGF knockdown is reported
255 to suppress the growth of endometriotic lesions, resulting in a significantly reduced
256 nerve fiber density in endometriosis and improvement in hyperalgesia [96].

257

258 Endometriotic lesions and their associated immune cells also secrete an array of pain
259 mediators, including PGE₂ [97], interleukins, histamine, and kinins [98]. These
260 mediators may act upon the terminals of primary sensory nerve fibers in or near
261 endometriotic lesions, causing pain. Extracellular acidosis due to either inflammation
262 or hypoxia in endometriosis [99] may also generate an acidic milieu, which in turn
263 may upregulate acid-sensitive ion channels (ASIC) expression in sensory neurons,
264 causing chemoreceptor hypersensitivity [100]. Sodium voltage-gated ion channels
265 (Na_v1.1-Na_v1.9) may be involved in EAP as well via estrogen-induced nociceptor
266 activation [95, 101].

267

268 Adenosine 5'-triphosphate (ATP), an intracellular energy source, is released from

269 cells during tissue stress, damage, or inflammation [102], and may also be released by

270 endometriotic lesions. Sensory neurons express receptors for ATP, known as P2X

271 purinoreceptors or P2XRs, which are ionotropic/ligand-gated ion channels distributed

272 throughout the CNS and PNS [103]. P2XR3 expression is elevated in the peritoneal

273 tissue of women with chronic pelvic pain, with and without endometriosis [95]. P2XR

274 activation by ATP on nociceptors initiates pain signals from damaged tissues [103]

275 and may mediate visceral hyperalgesia [104]. Since estrogen is produced locally in

276 endometriotic lesions [105], it may facilitate P2XR3 expression in sensory nerves

277 [106], eliciting heightened pain perception. Thus, there is a high likelihood that

278 P2XR3 may also be involved in EAP.

279

280 N-methyl-D-aspartic acid receptors (NMDARs) are known to be involved in central

281 sensitization [107] and in increasing neurotransmitter release triggered by BDNF

282 [108]. Activation of NMDARs releases SP to elicit pain [109]. While their role in

283 EAP is still unclear, we have shown that both levo-tetrahydropalmatine and valproic

284 acid treatments significantly reduced the expression of NMDAR1 in DRG in

285 conjunction with improved generalized hyperalgesia in rats with induced

286 endometriosis [110], suggesting they are also likely to be involved in EAP.

287

288 Women with EAP display increased levels of glutamine in the brain [111] and of

endocannabinoids in the blood as well as reduced expression of cannabinoid receptor 1 (CB1) in endometriotic lesions [112]. Groups II and III metabotropic glutamate receptors (mGlu2/3) [113, 114] and CB1 [115, 116] are known to play an inhibitory role in pain, possibly by blocking TRPV1-induced activation of nociceptors. Additionally, members of potassium channel family, KCNK2 (TREK-1) and KCNK4 (TRAAK) are expressed in a subset of C-fiber nociceptors and can be modulated by noxious heat (ca. 40°C) and pressure [117]. Similarly, KCNK18 (TRESK), a primary contributor to the excitatory effects of sanshool in sensory neurons [118], may be critical in noxious sensation. Whether or not they are involved in EAP remains to be further investigated.

In view of these discussions, we made a list of possible perpetrators suspected to be involved in the generation of EAP (Table 4). Endometriotic lesions may impact on the behaviors of nerve fibers through the release of NTFs and also pain mediators, as summarized in Figure 2, resulting in neuroplasticity and neuropathic pain-like symptoms.

(Table 4 and Figure 2 about here)

Nerve fibers may also impact on endometriotic lesions

So far almost all studies published so far on nerve fibers in endometriosis has focused lopsidedly on the distribution, density, and types of nerve fibers in endometriosis, and, in recent years, on NTFs secreted by endometriotic lesions. A tacit assumption underlying these studies is that endometriotic lesions exert, rather unidirectionally, a neurogenetic effect on the nervous systems, resulting in increased density of nerve fibers. However, as we have shown fairly recently, endometriotic, as well as adenomyotic, lesions can be viewed as wounds that undergo repeated injury and repair [19-21, 119]. As such, neuromediators are bound to be involved, as in wound healing [22, 120] (Table 5). Indeed, it is well documented that local denervation causes delay in wound healing [121, 122]. Possible mechanisms for retarded wound healing include (1) neuropeptides are depleted in denervated tissues, which minimize their trophic effects and reduce cell proliferation [121]; (2) significantly less inflammatory cells, particularly macrophages, in the wound site, reduce chemotaxis in denervated wounds [122]; and (3) reduced microvascular responses in denervated tissues result from the desensitization of vascular smooth muscle [120].

(Table 5 about here)

Indeed, a mixture of sensory, sympathetic and parasympathetic nerves fibers are shown to play important roles in tumorigenesis of many types of cancers, which were associated with inflammation, angiogenesis, proliferation and innervations [24,

123-127], as in endometriosis [128]. Therefore, it is plausible that, in analogous to both malignant tumor and wound healing, the relationship between endometriotic lesions and nerve fibers is very likely to be bidirectional, in that nerve fibers and endometriotic lesions engage active cross-talks, and nerve fibers also promote the development of endometriosis and result in pain (Figure 2).

The intralesional nerve fibers may also secrete fractalkine is a well-characterized neuronal chemokine, and has a single and specific receptor CX3CR1, which has been reported to be expressed in endometriotic lesions [129]. While an earlier study reported lower fractalkine levels in the PF of women with endometriosis, recent studies reported a higher secretion and expression in endometriotic stromal cells [129] and intralesional nerve fibers [130], which may be responsible for the alternatively activation of macrophages in endometriosis [129]. Pancreatic cancer cells expressing CX3CR1 are found to have increased migratory and adhesive capability to neuronal cells [131]. Whether fractalkine has a similar effect on endometriotic lesions will await further investigation.

In particular, norepinephrine released by nerve fibers or the CNS due to chronic exposure to stressors like pain itself may promote the growth of endometriotic lesions through adrenergic receptor $\beta 2$ (ADRB2)-mediated proliferation and angiogenesis, as ADRB2 is expressed in endometriosis [132], very similar to cancer [133]. SP, secreted

by afferent sensory neurons [134] and detected in sensory nerve fibers close to endometriotic lesions (see Table 2), promotes proliferation of endometriotic stromal cells through neurokinin1receptor (NK1R) (Yan et al., unpublished data), which is expressed in endometriosis [135]. While the roles of CGRP and other neuropeptides in the development of endometriosis are completely unclear, it has been reported that CGRP and VIP promote the invasiveness and motility of cancer cells [136]. In addition, glial-derived NTF (GDNF) and artemin also have been shown to increase the invasiveness of cancer cells [137]. Whether these NTFs and neuropeptides have similar effect on endometriotic cells warrants further research.

Consistent with this notion, our recent animal experimentation shows that denervation, either chemically or surgically, results in reduced size of endometriotic lesions and improved pain behavior in mice with induced endometriosis (Yan et al., unpublished data). Certain molecules, such as SP, secreted by sensory neurons promote proliferation and invasiveness in endometriotic stromal and epithelial cells (Yan et al., unpublished data). While the exact molecular mechanisms underlying the nerve fiber-endometriotic lesion cross-talk still await more research, it seems certain that the two parties interact closely to promote the development of endometriosis.

Conclusions

Accumulating evidence strongly indicates that increased neural density in endometriotic lesions is increased. Endometriotic lesions secrete NTFs that promote intralesional neural sprouting, hypertrophy and induce neural remodeling. In addition, hyperinnervation in eutopic endometrium also has been demonstrated but this hyperinnervation does not seem to be present exclusively in endometriosis. Consequently, the prospect of using endometrial nerve fiber density for diagnostic purpose faces serious challenges, particularly so when it comes to *differential diagnosis*. While few NTFs secreted by endometriotic lesions have been identified, it seems certain that the list of these NTFs is by no means exhaustive. Moreover, immune cells infiltrated into the endometriotic lesions may also secrete NTFs, and other molecules such as TXA₂ may also serve as NTFs that promote neural sprouting, hypertrophy or remodeling, further promoting intralesional neuroplasticity.

As endometriotic lesions are fundamentally wounds undergoing ReTAIR [19-21], we postulate that NTFs, neurotransmitters and neuropeptides secreted by intralesional nerve fibers should also affect the behavior and functions of endometriotic lesions. Consequently, nerve fibers and endometriotic lesions may engage active communication, facilitating the development of endometriosis. That is, nerve fibers and endometriotic lesions are truly partners in crime in inflicting pains in women with endometriosis. Our preliminary in vivo and in vitro data appear to support this notion. While the detailed mechanisms still warrant more research, this cross-talk adds another layer of complexity when trying to pinpoint the cause and effect in EAP, since

the two parties could be both the cause and the effect. In addition, there may well be other aiders and abettors, yet to be identified and indicted, in committing the crime that await to be rounded up in the future. Regardless, this perspective should help to unravel the relationship between endometriotic lesions and nerve fibers, sort out the mechanisms underlying EAP, and may provide novel ways for medical intervention.

Acknowledgment

This research was supported in part by grants 81270676 (SWG), 81471434 (SWG), 81530040 (SWG), and 81370695 (XSL) from the National Science Foundation of China, and grant 2013ZYJB0019 (XSL) from Shanghai Municipal Commission of Health and Family Planning.

References

- (1) Guo SW. Emerging drugs for endometriosis. Expert Opin Emerg Drugs. 2008;13:547-71.
- (2) Olive DL, Lindheim SR, Pritts EA. New medical treatments for endometriosis. Best Pract Res Clin Obstet Gynaecol. 2004;18:319-28.
- (3) Giudice LC, Kao LC. Endometriosis. Lancet (London, England). 2004;364:1789-99.
- (4) Nnoaham KE, Hummelshoj L, Webster P, d'Hooghe T, de Cicco Nardone F, de

416 Cicco Nardone C, et al. Impact of endometriosis on quality of life and work
 417 productivity: a multicenter study across ten countries. *Fertility and sterility*.
 418 2011;96:366-73 e8.
 419 (5) Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I, et al.
 420 The burden of endometriosis: costs and quality of life of women with endometriosis
 421 and treated in referral centres. *Human reproduction* (Oxford, England).
 422 2012;27:1292-9.
 423 (6) Howard FM. Endometriosis and mechanisms of pelvic pain. *Journal of minimally*
 424 *invasive gynecology*. 2009;16:540-50.
 425 (7) Whiteside JL, Falcone T. Endometriosis-related pelvic pain: what is the evidence?
 426 *Clinical obstetrics and gynecology*. 2003;46:824-30.
 427 (8) Vercellini P. Endometriosis: what a pain it is. *Semin Reprod Endocrinol*.
 428 1997;15:251-61.
 429 (9) Marana R, Muzii L, Caruana P, Dell'Acqua S, Mancuso S. Evaluation of the
 430 correlation between endometriosis extent, age of the patients and associated
 431 symptomatology. *Acta Eur Fertil*. 1991;22:209-12.
 432 (10) Fedele L, Bianchi S, Bocciolone L, Di Nola G, Parazzini F. Pain symptoms
 433 associated with endometriosis. *Obstetrics and gynecology*. 1992;79:767-9.
 434 (11) Fedele L, Parazzini F, Bianchi S, Arcaini L, Candiani GB. Stage and localization
 435 of pelvic endometriosis and pain. *Fertility and sterility*. 1990;53:155-8.
 436 (12) Muzii L, Marana R, Pedulla S, Catalano GF, Mancuso S. Correlation between
 437 endometriosis-associated dysmenorrhea and the presence of typical or atypical

lesions. Fertility and sterility. 1997;68:19-22.

(13) (IASP) IAftSoP. IASP Taxonomy: Pain. 2015.

(14) Giudice LC. Clinical practice. Endometriosis. N Engl J Med. 2010;362:2389-98.

(15) He W, Liu X, Zhang Y, Guo SW. Generalized hyperalgesia in women with endometriosis and its resolution following a successful surgery. Reproductive sciences (Thousand Oaks, Calif). 2010;17:1099-111.

(16) As-Sanie S, Harris RE, Napadow V, Kim J, Neshewat G, Kairys A, et al. Changes in regional gray matter volume in women with chronic pelvic pain: a voxel-based morphometry study. Pain. 2012;153:1006-14.

(17) As-Sanie S, Kim J, Schmidt-Wilcke T, Sundgren PC, Clauw DJ, Napadow V, et al. Functional Connectivity Is Associated With Altered Brain Chemistry in Women With Endometriosis-Associated Chronic Pelvic Pain. J Pain. 2015.

(18) Chen Y, Zhu B, Zhang H, Ding D, Liu X, Guo SW. Possible Loss of GABAergic Inhibition in Mice With Induced Adenomyosis and Treatment With Epigallocatechin-3-Gallate Attenuates the Loss With Improved Hyperalgesia. Reproductive sciences (Thousand Oaks, Calif). 2014;21:869-82.

(19) Ding D, Liu X, Duan J, Guo SW. Platelets are an unindicted culprit in the development of endometriosis: clinical and experimental evidence. Human reproduction (Oxford, England). 2015;30:812-32.

(20) Guo SW, Ding D, Shen M, Liu X. Dating Endometriotic Ovarian Cysts Based on the Content of Cyst Fluid and its Potential Clinical Implications. Reproductive sciences (Thousand Oaks, Calif). 2015;22:873-83.

- 1 460 (21) Zhang Q, Duan J, Olson M, Fazleabas A, Guo SW. Cellular changes consistent
2
3 461 with epithelial-mesenchymal transition and fibroblast-to-myofibroblast
4
5 462 transdifferentiation in the progression of experimental endometriosis in baboons.
6
7
8 463 Reprod Sci. 2016;In press.
- 10
11 464 (22) Laverdet B, Danigo A, Girard D, Magy L, Demiot C, Desmouliere A. Skin
12
13 465 innervation: important roles during normal and pathological cutaneous repair. Histol
14
15 466 Histopathol. 2015;30:875-92.
- 17
18 467 (23) Purves D. Neuroscience. 3rd ed. Sunderland, Mass.: Sinauer Associates; 2004.
- 20
21 468 (24) Hanoun M, Maryanovich M, Arnal-Estape A, Frenette PS. Neural regulation of
22
23 469 hematopoiesis, inflammation, and cancer. Neuron. 2015;86:360-73.
- 25
26 470 (25) Tulandi T, Chen MF, Al-Took S, Watkin K. A study of nerve fibers and
27
28 471 histopathology of postsurgical, postinfectious, and endometriosis-related adhesions.
29
30 472 Obstetrics and gynecology. 1998;92:766-8.
- 32
33 473 (26) Tulandi T, Felemban A, Chen MF. Nerve fibers and histopathology of
34
35 474 endometriosis-harboring peritoneum. The Journal of the American Association of
36
37 475 Gynecologic Laparoscopists. 2001;8:95-8.
- 39
40 476 (27) Al-Fozan H, Bakare S, Chen MF, Tulandi T. Nerve fibers in ovarian dermoid
41
42 477 cysts and endometriomas. Fertility and sterility. 2004;82:230-1.
- 44
45 478 (28) Anaf V, Simon P, El Nakadi I, Fayt I, Buxant F, Simonart T, et al. Relationship
46
47 479 between endometriotic foci and nerves in rectovaginal endometriotic nodules. Human
48
49 480 reproduction (Oxford, England). 2000;15:1744-50.
- 51
52 481 (29) Quinn MJ, Kirk N. Differences in uterine innervation at hysterectomy. American

journal of obstetrics and gynecology. 2002;187:1515-9; discussion 9-20.

(30) Quinn M. Endometriosis: the consequence of neurological dysfunction? Medical hypotheses. 2004;63:602-8.

(31) Quinn M, Kirk N. Uterosacral nerve fibre proliferation in parous endometriosis. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology. 2004;24:189-90.

(32) Quinn MJ. Endometriosis: the consequence of uterine denervation-reinnervation. Archives of gynecology and obstetrics. 2011;284:1423-9.

(33) Berkley KJ, Dmitrieva N, Curtis KS, Papka RE. Innervation of ectopic endometrium in a rat model of endometriosis. Proceedings of the National Academy of Sciences of the United States of America. 2004;101:11094-8.

(34) Berkley KJ, Rapkin AJ, Papka RE. The pains of endometriosis. Science. 2005;308:1587-9.

(35) Tokushige N, Markham R, Russell P, Fraser IS. Nerve fibres in peritoneal endometriosis. Human reproduction (Oxford, England). 2006;21:3001-7.

(36) Al-Jefout M, Andreadis N, Tokushige N, Markham R, Fraser I. A pilot study to evaluate the relative efficacy of endometrial biopsy and full curettage in making a diagnosis of endometriosis by the detection of endometrial nerve fibers. Am J Obstet Gynecol. 2007;197:578 e1-4.

(37) Al-Jefout M, Dezarnaulds G, Cooper M, Tokushige N, Luscombe GM, Markham R, et al. Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: a double blind study. Human reproduction (Oxford, England).

2009;24:3019-24.

(38) Tokushige N, Markham R, Russell P, Fraser IS. High density of small nerve fibres in the functional layer of the endometrium in women with endometriosis. *Human reproduction* (Oxford, England). 2006;21:782-7.

(39) Tokushige N, Markham R, Russell P, Fraser IS. Different types of small nerve fibers in eutopic endometrium and myometrium in women with endometriosis. *Fertility and sterility*. 2007;88:795-803.

(40) Tokushige N, Markham R, Russell P, Fraser IS. Effects of hormonal treatment on nerve fibers in endometrium and myometrium in women with endometriosis. *Fertility and sterility*. 2008;90:1589-98.

(41) Tokushige N, Markham R, Russell P, Fraser IS. Effect of progestogens and combined oral contraceptives on nerve fibers in peritoneal endometriosis. *Fertility and sterility*. 2009;92:1234-9.

(42) Wang G, Tokushige N, Russell P, Dubinovsky S, Markham R, Fraser IS. Neuroendocrine cells in eutopic endometrium of women with endometriosis. *Human reproduction* (Oxford, England). 2010;25:387-91.

(43) Miller EJ, Fraser IS. The importance of pelvic nerve fibers in endometriosis. *Women's health* (London, England). 2015.

(44) Quinn M. Uterine innervation in adenomyosis. *Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology*. 2007;27:287-91.

(45) Mechsner S, Kaiser A, Kopf A, Gericke C, Ebert A, Bartley J. A pilot study to

1 526 evaluate the clinical relevance of endometriosis-associated nerve fibers in peritoneal
2
3 527 endometriotic lesions. Fertility and sterility. 2009;92:1856-61.
4
5
6 528 (46) Wang G, Tokushige N, Markham R, Fraser IS. Rich innervation of deep
7
8 529 infiltrating endometriosis. Human reproduction (Oxford, England). 2009;24:827-34.
9
10
11 530 (47) Bokor A, Kyama CM, Vercruysse L, Fassbender A, Gevaert O, Vodolazkaia A,
12
13 531 et al. Density of small diameter sensory nerve fibres in endometrium: a semi-invasive
14
15 532 diagnostic test for minimal to mild endometriosis. Human reproduction (Oxford,
16
17 533 England). 2009;24:3025-32.
18
19
20
21
22 534 (48) Zhang X, Lu B, Huang X, Xu H, Zhou C, Lin J. Endometrial nerve fibers in
23
24 535 women with endometriosis, adenomyosis, and uterine fibroids. Fertility and sterility.
25
26 536 2009;92:1799-801.
27
28
29
30
31 537 (49) Zhang X, Lu B, Huang X, Xu H, Zhou C, Lin J. Innervation of endometrium and
32
33 538 myometrium in women with painful adenomyosis and uterine fibroids. Fertility and
34
35 539 sterility. 2010;94:730-7.
36
37
38
39 540 (50) Zhang X, Yao H, Huang X, Lu B, Xu H, Zhou C. Nerve fibres in ovarian
40
41 541 endometriotic lesions in women with ovarian endometriosis. Human reproduction
42
43 542 (Oxford, England). 2010;25:392-7.
44
45
46
47 543 (51) Donnez O, Soares M, Defrere S, Dehoux JP, van Langendonckt A, Donnez J, et
48
49 544 al. Nerve fiber density in deep nodular endometriotic lesions induced in a baboon
50
51 545 experimental model. Fertility and sterility. 2013;100:1144-50.
52
53
54
55 546 (52) Barcena de Arellano ML, Oldeweme J, Arnold J, Schneider A, Mechsner S.
56
57 547 Remodeling of estrogen-dependent sympathetic nerve fibers seems to be disturbed in
58
59
60
61
62
63
64
65

adenomyosis. *Fertility and sterility*. 2013;100:801-9.

(53) Anaf V, Chapron C, El Nakadi I, De Moor V, Simonart T, Noel JC. Pain, mast cells, and nerves in peritoneal, ovarian, and deep infiltrating endometriosis. *Fertility and sterility*. 2006;86:1336-43.

(54) Atwal G, du Plessis D, Armstrong G, Slade R, Quinn M. Uterine innervation after hysterectomy for chronic pelvic pain with, and without, endometriosis. *American journal of obstetrics and gynecology*. 2005;193:1650-5.

(55) Barcena de Arellano ML, Arnold J, Sacher F, Blochle M, Staube M, Bartley J, et al. Eutopic endometrium from women with endometriosis does not exhibit neurotrophic properties. *Journal of neuroimmunology*. 2012;249:49-55.

(56) Di Spiezio Sardo A, Florio P, Fernandez LM, Guerra G, Spinelli M, Di Carlo C, et al. The potential role of endometrial nerve fibers in the pathogenesis of pain during endometrial biopsy at office hysteroscopy. *Reproductive sciences (Thousand Oaks, Calif)*. 2015;22:124-31.

(57) Kelm Junior AR, Lancellotti CL, Donadio N, Auge AP, Lima SM, Aoki T, et al. Nerve fibers in uterosacral ligaments of women with deep infiltrating endometriosis. *Journal of reproductive immunology*. 2008;79:93-9.

(58) Tokushige N, Russell P, Black K, Barrera H, Dubinovsky S, Markham R, et al. Nerve fibers in ovarian endometriomas. *Fertility and sterility*. 2010;94:1944-7.

(59) Possover M, Tersiev P, Angelov DN. Comparative study of the neuropeptide-Y sympathetic nerves in endometriotic involved and noninvolved sacrouterine ligaments in women with pelvic endometriosis. *Journal of minimally invasive gynecology*.

2009;16:340-3.

(60) Ferrero S, Haas S, Remorgida V, Camerini G, Fulcheri E, Ragni N, et al. Loss of sympathetic nerve fibers in intestinal endometriosis. Fertility and sterility. 2010;94:2817-9.

(61) Arnold J, Barcena de Arellano ML, Ruster C, Vercellino GF, Chiantera V, Schneider A, et al. Imbalance between sympathetic and sensory innervation in peritoneal endometriosis. Brain, behavior, and immunity. 2012;26:132-41.

(62) Mechsner S, Schwarz J, Thode J, Loddenkemper C, Salomon DS, Ebert AD. Growth-associated protein 43-positive sensory nerve fibers accompanied by immature vessels are located in or near peritoneal endometriotic lesions. Fertil Steril. 2007;88:581-7.

(63) Wang G, Tokushige N, Russell P, Dubinovsky S, Markham R, Fraser IS. Hyperinnervation in intestinal deep infiltrating endometriosis. Journal of minimally invasive gynecology. 2009;16:713-9.

(64) Morotti M, Vincent K, Brawn J, Zondervan KT, Becker CM. Peripheral changes in endometriosis-associated pain. Hum Reprod Update. 2014;20:717-36.

(65) Barcena de Arellano ML, Wagner MF, Oldeweme J, Arnold J, Ebert A, Schneider A, et al. Neurotrophin expression is not affected in uteri of women with adenomyosis. Journal of molecular neuroscience : MN. 2012;47:495-504.

(66) Herrick SE, Mutsaers SE, Ozua P, Sulaiman H, Omer A, Boulos P, et al. Human peritoneal adhesions are highly cellular, innervated, and vascularized. J Pathol. 2000;192:67-72.

- 1 592 (67) Leslie C, Ma T, McElhinney B, Leake R, Stewart CJ. Is the detection of
2
3 593 endometrial nerve fibers useful in the diagnosis of endometriosis? International
4
5
6 594 journal of gynecological pathology : official journal of the International Society of
7
8
9 595 Gynecological Pathologists. 2013;32:149-55.
- 10
11 596 (68) Neis KJ, Neis F. Chronic pelvic pain: cause, diagnosis and therapy from a
12
13
14 597 gynaecologist's and an endoscopist's point of view. Gynecol Endocrinol.
15
16
17 598 2009;25:757-61.
- 18
19
20 599 (69) Gupta D, Hull ML, Fraser I, Miller L, Bossuyt PM, Johnson N, et al. Endometrial
21
22
23 600 biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst
24
25
26 601 Rev. 2016;4:CD012165.
- 27
28 602 (70) Arnold J, Vercellino GF, Chiantera V, Schneider A, Mechsner S, Barcena de
29
30
31 603 Arellano ML. Neuroimmunomodulatory alterations in non-lesional peritoneum close
32
33
34 604 to peritoneal endometriosis. Neuroimmunomodulation. 2013;20:9-18.
- 35
36 605 (71) Huber AB, Kolodkin AL, Ginty DD, Cloutier JF. Signaling at the growth cone:
37
38
39 606 ligand-receptor complexes and the control of axon growth and guidance. Annu Rev
40
41
42 607 Neurosci. 2003;26:509-63.
- 43
44 608 (72) Liang Y, Wang W, Huang J, Tan H, Liu T, Shang C, et al. Potential Role of
45
46
47 609 Semaphorin 3A and Its Receptors in Regulating Aberrant Sympathetic Innervation in
48
49
50 610 Peritoneal and Deep Infiltrating Endometriosis. PloS one. 2015;10:e0146027.
- 51
52
53 611 (73) Asante A, Taylor RN. Endometriosis: the role of neuroangiogenesis. Annual
54
55
56 612 review of physiology. 2011;73:163-82.
- 57
58 613 (74) Boyd JG, Gordon T. Neurotrophic factors and their receptors in axonal
59
60

regeneration and functional recovery after peripheral nerve injury. *Mol Neurobiol.* 2003;27:277-324.

(75) Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci.* 2003;4:299-309.

(76) Zhang YH, Nicol GD. NGF-mediated sensitization of the excitability of rat sensory neurons is prevented by a blocking antibody to the p75 neurotrophin receptor. *Neurosci Lett.* 2004;366:187-92.

(77) Choi YS, Cho S, Lim KJ, Jeon YE, Yang HI, Lee KE, et al. Effects of LNG-IUS on nerve growth factor and its receptors expression in patients with adenomyosis. *Growth Factors.* 2010;28:452-60.

(78) Tarjanne S, Ng CH, Manconi F, Arola J, Mentula M, Maneck B, et al. Use of hormonal therapy is associated with reduced nerve fiber density in deep infiltrating, rectovaginal endometriosis. *Acta obstetricia et gynecologica Scandinavica.* 2015;94:693-700.

(79) Apfel SC. Neurotrophic factors and pain. *Clin J Pain.* 2000;16:S7-11.

(80) Kajitani T, Maruyama T, Asada H, Uchida H, Oda H, Uchida S, et al. Possible involvement of nerve growth factor in dysmenorrhea and dyspareunia associated with endometriosis. *Endocrine journal.* 2013;60:1155-64.

(81) Barcena de Arellano ML, Arnold J, Vercellino F, Chiantera V, Schneider A, Mechsner S. Overexpression of nerve growth factor in peritoneal fluid from women with endometriosis may promote neurite outgrowth in endometriotic lesions. *Fertility and sterility.* 2011;95:1123-6.

- 636 (82) Barcena de Arellano ML, Arnold J, Lang H, Vercellino GF, Chiantera V,
637 Schneider A, et al. Evidence of neurotrophic events due to peritoneal endometriotic
638 lesions. *Cytokine*. 2013;62:253-61.
- 639 (83) Browne AS, Yu J, Huang RP, Francisco AM, Sidell N, Taylor RN. Proteomic
640 identification of neurotrophins in the eutopic endometrium of women with
641 endometriosis. *Fertility and sterility*. 2012;98:713-9.
- 642 (84) Wessels JM, Kay VR, Leyland NA, Agarwal SK, Foster WG. Assessing
643 brain-derived neurotrophic factor as a novel clinical marker of endometriosis. *Fertil*
644 *Steril*. 2015.
- 645 (85) Benowitz LI, Routtenberg A. GAP-43: an intrinsic determinant of neuronal
646 development and plasticity. *Trends Neurosci*. 1997;20:84-91.
- 647 (86) Barcena de Arellano ML, Arnold J, Vercellino GF, Chiantera V, Ebert AD,
648 Schneider A, et al. Influence of nerve growth factor in endometriosis-associated
649 symptoms. *Reprod Sci*. 2011;18:1202-10.
- 650 (87) Alvarez P, Chen X, Hendrich J, Irwin JC, Green PG, Giudice LC, et al. Ectopic
651 uterine tissue as a chronic pain generator. *Neuroscience*. 2012;225:269-82.
- 652 (88) Sumimoto S, Muramatsu R, Yamashita T. Thromboxane A2 stimulates neurite
653 outgrowth in cerebral cortical neurons via mitogen activated protein kinase signaling.
654 *Brain Res*. 2015;1594:46-51.
- 655 (89) Guo S-W, Du Y, Liu X. Endometriosis-Derived Stromal Cells Secrete
656 Thrombin and Thromboxane A2, Inducing Platelet Activation. . *Reprod Sci*. 2016;In
657 press.

- 658 (90) McMahon SB. NGF as a mediator of inflammatory pain. *Philos Trans R Soc Lond B Biol Sci.* 1996;351:431-40.
- 660 (91) Nie J, Liu X, Guo SW. Immunoreactivity of oxytocin receptor and transient receptor potential vanilloid type 1 and its correlation with dysmenorrhea in adenomyosis. *American journal of obstetrics and gynecology.* 2010;202:346 e1-8.
- 663 (92) Rocha MG, e Silva JC, Ribeiro da Silva A, Candido Dos Reis FJ, Nogueira AA, Poli-Neto OB. TRPV1 expression on peritoneal endometriosis foci is associated with chronic pelvic pain. *Reproductive sciences (Thousand Oaks, Calif).* 2011;18:511-5.
- 666 (93) Liu J, Liu X, Duan K, Zhang Y, Guo SW. The expression and functionality of transient receptor potential vanilloid 1 in ovarian endometriomas. *Reproductive sciences (Thousand Oaks, Calif).* 2012;19:1110-24.
- 669 (94) Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, et al. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature.* 2001;411:957-62.
- 672 (95) Greaves E, Grieve K, Horne AW, Saunders PT. Elevated peritoneal expression and estrogen regulation of nociceptive ion channels in endometriosis. *The Journal of clinical endocrinology and metabolism.* 2014;99:E1738-43.
- 675 (96) Chen Y, Li D, Zhang Z, Takushige N, Kong BH, Wang GY. Effect of siRNA against beta-NGF on nerve fibers of a rat model with endometriosis. *Reproductive sciences (Thousand Oaks, Calif).* 2014;21:329-39.
- 678 (97) Wu MH, Lu CW, Chuang PC, Tsai SJ. Prostaglandin E2: the master of endometriosis? *Exp Biol Med (Maywood).* 2010;235:668-77.

- 680 (98) Anaf V, Simon P, El Nakadi I, Fayt I, Simonart T, Buxant F, et al. Hyperalgesia,
681 nerve infiltration and nerve growth factor expression in deep adenomyotic nodules,
682 peritoneal and ovarian endometriosis. Human reproduction (Oxford, England).
683 2002;17:1895-900.
- 684 (99) Wu MH, Chen KF, Lin SC, Lgu CW, Tsai SJ. Aberrant expression of leptin in
685 human endometriotic stromal cells is induced by elevated levels of hypoxia inducible
686 factor-1alpha. Am J Pathol. 2007;170:590-8.
- 687 (100) Liu X, He L, Dinger B, Fidone SJ. Chronic hypoxia-induced acid-sensitive ion
688 channel expression in chemoafferent neurons contributes to chemoreceptor
689 hypersensitivity. Am J Physiol Lung Cell Mol Physiol. 2011;301:L985-92.
- 690 (101) McKinnon BD, Bertschi D, Bersinger NA, Mueller MD. Inflammation and
691 nerve fiber interaction in endometriotic pain. Trends in endocrinology and
692 metabolism: TEM. 2015;26:1-10.
- 693 (102) Burnstock G. P2X receptors in sensory neurones. Br J Anaesth.
694 2000;84:476-88.
- 695 (103) Chen CC, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN. A
696 P2X purinoceptor expressed by a subset of sensory neurons. Nature.
697 1995;377:428-31.
- 698 (104) Xu GY, Shenoy M, Winston JH, Mittal S, Pasricha PJ. P2X receptor-mediated
699 visceral hyperalgesia in a rat model of chronic visceral hypersensitivity. Gut.
700 2008;57:1230-7.
- 701 (105) Attar E, Bulun SE. Aromatase and other steroidogenic genes in endometriosis:

translational aspects. Hum Reprod Update. 2006;12:49-56.

(106) Fan J, Yu LH, Zhang Y, Ni X, Ma B, Burnstock G. Estrogen altered visceromotor reflex and P2X(3) mRNA expression in a rat model of colitis. Steroids. 2009;74:956-62.

(107) Woolf CJ, Thompson SW. The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. Pain. 1991;44:293-9.

(108) Kerr BJ, Bradbury EJ, Bennett DL, Trivedi PM, Dassan P, French J, et al. Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord. J Neurosci. 1999;19:5138-48.

(109) Liu H, Mantyh PW, Basbaum AI. NMDA-receptor regulation of substance P release from primary afferent nociceptors. Nature. 1997;386:721-4.

(110) Zhao T, Liu X, Zhen X, Guo SW. Levo-tetrahydropalmatine retards the growth of ectopic endometrial implants and alleviates generalized hyperalgesia in experimentally induced endometriosis in rats. Reproductive sciences (Thousand Oaks, Calif). 2011;18:28-45.

(111) As-Sanie S, Kim J, Schmidt-Wilcke T, Sundgren PC, Clauw DJ, Napadow V, et al. Functional Connectivity is Associated With Altered Brain Chemistry in Women With Endometriosis-Associated Chronic Pelvic Pain. J Pain. 2016;17:1-13.

(112) Sanchez AM, Cioffi R, Vigano P, Candiani M, Verde R, Piscitelli F, et al. Elevated Systemic Levels of Endocannabinoids and Related Mediators Across the

Menstrual Cycle in Women With Endometriosis. *Reprod Sci*. 2016.

(113) Govea RM, Zhou S, Carlton SM. Group III metabotropic glutamate receptors and transient receptor potential vanilloid 1 co-localize and interact on nociceptors. *Neuroscience*. 2012;217:130-9.

(114) Acher F, Goudet C. Therapeutic potential of group III metabotropic glutamate receptor ligands in pain. *Curr Opin Pharmacol*. 2015;20:64-72.

(115) Dmitrieva N, Nagabukuro H, Resuehr D, Zhang G, McAllister SL, McGinty KA, et al. Endocannabinoid involvement in endometriosis. *Pain*. 2010;151:703-10.

(116) Wang ZY, McDowell T, Wang P, Alvarez R, Gomez T, Bjorling DE. Activation of CB1 inhibits NGF-induced sensitization of TRPV1 in adult mouse afferent neurons. *Neuroscience*. 2014;277:679-89.

(117) Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. *Cell*. 2009;139:267-84.

(118) Bautista DM, Sigal YM, Milstein AD, Garrison JL, Zorn JA, Tsuruda PR, et al. Pungent agents from Szechuan peppers excite sensory neurons by inhibiting two-pore potassium channels. *Nat Neurosci*. 2008;11:772-9.

(119) Shen M, Liu X, Zhang H, Guo SW. Transforming Growth Factor β 1 Signaling Coincides with Platelet-Induced Epithelial-Mesenchymal Transition and Fibroblast-to-Myofibroblast Transdifferentiation in Drive the Development of Adenomyosis in Mice. *Human reproduction* (Oxford, England). 2016; 31:355-69.

(120) Barker AR, Rosson GD, Dellon AL. Wound healing in denervated tissue. *Ann Plast Surg*. 2006;57:339-42.

- 1 746 (121) Engin C, Demirkan F, Ayhan S, Atabay K, Baran NK. Delayed effect of
2
3 747 denervation on wound contraction in rat skin. *Plast Reconstr Surg*. 1996;98:1063-7.
4
5
6 748 (122) Richards AM, Floyd DC, Terenghi G, McGrouther DA. Cellular changes in
7
8 749 denervated tissue during wound healing in a rat model. *Br J Dermatol*.
9
10 750 1999;140:1093-9.
11
12
13 751 (123) Entschladen F, Drell TLt, Lang K, Joseph J, Zaenker KS. Tumour-cell
14
15 752 migration, invasion, and metastasis: navigation by neurotransmitters. *Lancet Oncol*.
16
17 753 2004;5:254-8.
18
19
20
21
22 754 (124) Ayala GE, Dai H, Tahir SA, Li R, Timme T, Ittmann M, et al. Stromal
23
24 755 antiapoptotic paracrine loop in perineural invasion of prostatic carcinoma. *Cancer*
25
26 756 *Res*. 2006;66:5159-64.
27
28
29
30
31 757 (125) Erin N, Barkan GA, Clawson GA. Vagus nerve regulates breast cancer
32
33 758 metastasis to the adrenal gland. *Anticancer Res*. 2013;33:3675-82.
34
35
36 759 (126) Magnon C, Hall SJ, Lin J, Xue X, Gerber L, Freedland SJ, et al. Autonomic
37
38 760 nerve development contributes to prostate cancer progression. *Science*.
39
40 761 2013;341:1236361.
41
42
43
44 762 (127) Zhao CM, Hayakawa Y, Kodama Y, Muthupalani S, Westphalen CB, Andersen
45
46 763 GT, et al. Denervation suppresses gastric tumorigenesis. *Sci Transl Med*.
47
48 764 2014;6:250ra115.
49
50
51
52 765 (128) Guo SW. Endometriosis and ovarian cancer: potential benefits and harms of
53
54 766 screening and risk-reducing surgery. *Fertility and sterility*. 2015;104:813-30.
55
56
57
58 767 (129) Wang Y, Fu Y, Xue S, Ai A, Chen H, Lyu Q, et al. The M2 polarization of
59
60
61
62
63
64
65

macrophage induced by fractalkine in the endometriotic milieu enhances invasiveness
of endometrial stromal cells. *Int J Clin Exp Pathol.* 2014;7:194-203.

(130) Chen S, Xie W, Strong JA, Jiang J, Zhang JM. Sciatic endometriosis induces
mechanical hypersensitivity, segmental nerve damage, and robust local inflammation
in rats. *Eur J Pain.* 2015.

(131) Marchesi F, Locatelli M, Solinas G, Erreni M, Allavena P, Mantovani A. Role
of CX3CR1/CX3CL1 axis in primary and secondary involvement of the nervous
system by cancer. *Journal of neuroimmunology.* 2010;224:39-44.

(132) Long Q, Liu X, Guo SW. Surgery Accelerates the Development of
Endometriosis in Mouse. *Am J Obstet Gynecol.* 2016;In press.

(133) Melhem-Bertrandt A, Sood AK. Adrenergic signaling and cancer: deciphering
the connections. *Cancer Biomark.* 2013;13:131-2.

(134) Holzer P. Local effector functions of capsaicin-sensitive sensory nerve endings:
involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides.
Neuroscience. 1988;24:739-68.

(135) McKinnon BD, Evers J, Bersinger NA, Mueller MD. Induction of the
neurokinin 1 receptor by TNFalpha in endometriotic tissue provides the potential for
neurogenic control over endometriotic lesion growth. *The Journal of clinical
endocrinology and metabolism.* 2013;98:2469-77.

(136) Nagakawa O, Ogasawara M, Murata J, Fuse H, Saiki I. Effect of prostatic
neuropeptides on migration of prostate cancer cell lines. *Int J Urol.* 2001;8:65-70.

(137) Funahashi H, Okada Y, Sawai H, Takahashi H, Matsuo Y, Takeyama H, et al.

790 The role of glial cell line-derived neurotrophic factor (GDNF) and integrins for
 791 invasion and metastasis in human pancreatic cancer cells. J Surg Oncol.
 792 2005;91:77-83.
 793 (138) Al-Jefout M, Andreadis N, Tokushige N, Markham R, Fraser I. A pilot study to
 794 evaluate the relative efficacy of endometrial biopsy and full curettage in making a
 795 diagnosis of endometriosis by the detection of endometrial nerve fibers. American
 796 journal of obstetrics and gynecology. 2007;197:578.e1-4.
 797 (139) Aghaey Meibody F, Mehdizadeh Kashi A, Zare Mirzaie A, Ghajarie Bani
 798 Amam M, Shariati Behbahani A, Zolali B, et al. Diagnosis of endometrial nerve fibers
 799 in women with endometriosis. Archives of gynecology and obstetrics.
 800 2011;284:1157-62.
 801 (140) Makari S SE, Railaite D R, Cizauskas A, Makari J. Endometrial nerve fibers in
 802 the diagnosis of endometriosis [Endometriumo nervu skaidulu reiksme diagnozuojant
 803 endometrioze]. Lietuvos akuserija ir ginekologija [Lithuanian Obstetrics and
 804 Gynaecology]. 2012;15:125-30.
 805 (141) Elgafor El Sharkwy IA. Combination of non-invasive and semi-invasive tests
 806 for diagnosis of minimal to mild endometriosis. Archives of gynecology and
 807 obstetrics. 2013;288:793-7.
 808 (142) Yadav G RG, Singh N, Radhika AG. Detection of endometrial nerve fibres- a
 809 novel technique to diagnose endometriosis. Journal of Endometriosis and Pelvic Pain
 810 Disorders. 2013;5:144-50.
 811 (143) Zevallos HB, McKinnon B, Tokushige N, Mueller MD, Fraser IS, Bersinger

812 NA. Detection of the pan neuronal marker PGP9.5 by immuno-histochemistry and
 813 quantitative PCR in eutopic endometrium from women with and without
 814 endometriosis. Archives of gynecology and obstetrics. 2015;291:85-91.
 815 (144) Newman TA, Bailey JL, Stocker LJ, Woo YL, Macklon NS, Cheong YC.
 816 Expression of neuronal markers in the endometrium of women with and those without
 817 endometriosis. Human reproduction (Oxford, England). 2013;28:2502-10.
 818 (145) Cetin C, Serdaroglu H, Tuzlali S. The importance of endometrial nerve fibers
 819 and macrophage cell count in the diagnosis of endometriosis. Iranian journal of
 820 reproductive medicine. 2013;11:405-14.
 821 (146) Olivier Donnez MS, Sylvie Defrère, Olivier Van Kerk, Anne Van
 822 Langendonckt, Jacques Donnez, Marie-Madeleine Dolmans, Sébastien Colette. Nerve
 823 fibers are absent in disease-free and eutopic endometrium, but present in
 824 endometriotic (especially deep) lesions. Journal of Endometriosis and Pelvic Pain
 825 Disorders. 2013;00:000-.
 826 (147) Ellett L, Readman E, Newman M, McIlwaine K, Villegas R, Jagasia N, et al.
 827 Are endometrial nerve fibres unique to endometriosis? A prospective case-control
 828 study of endometrial biopsy as a diagnostic test for endometriosis in women with
 829 pelvic pain. Human reproduction (Oxford, England). 2015;30:2808-15.
 830 (148) Wong H, Kang I, Dong XD, Christidis N, Ernberg M, Svensson P, et al.
 831 NGF-induced mechanical sensitization of the masseter muscle is mediated through
 832 peripheral NMDA receptors. Neuroscience. 2014;269:232-44.
 833 (149) Chen W, Walwyn W, Ennes HS, Kim H, McRoberts JA, Marvizon JC. BDNF

834 released during neuropathic pain potentiates NMDA receptors in primary afferent
 835 terminals. *Eur J Neurosci.* 2014;39:1439-54.
 836 (150) Hunt SP, Mantyh PW. The molecular dynamics of pain control. *Nat Rev*
 837 *Neurosci.* 2001;2:83-91.
 838 (151) Nagaraja AS, Dorniak PL, Sadaoui NC, Kang Y, Lin T, Armaiz-Pena G, et al.
 839 Sustained adrenergic signaling leads to increased metastasis in ovarian cancer via
 840 increased PGE2 synthesis. *Oncogene.* 2015.
 841 (152) Dib-Hajj SD, Yang Y, Black JA, Waxman SG. The Na(V)1.7 sodium channel:
 842 from molecule to man. *Nat Rev Neurosci.* 2013;14:49-62.
 843 (153) Li MQ, Li HP, Meng YH, Wang XQ, Zhu XY, Mei J, et al. Chemokine CCL2
 844 enhances survival and invasiveness of endometrial stromal cells in an autocrine
 845 manner by activating Akt and MAPK/Erk1/2 signal pathway. *Fertility and sterility.*
 846 2012;97:919-29.
 847 (154) Kao DJ, Li AH, Chen JC, Luo RS, Chen YL, Lu JC, et al. CC chemokine ligand
 848 2 upregulates the current density and expression of TRPV1 channels and Nav1.8
 849 sodium channels in dorsal root ganglion neurons. *J Neuroinflammation.* 2012;9:189.
 850 (155) Alloui A, Zimmermann K, Mamet J, Duprat F, Noel J, Chemin J, et al.
 851 TREK-1, a K⁺ channel involved in polymodal pain perception. *EMBO J.*
 852 2006;25:2368-76.
 853 (156) Noel J, Zimmermann K, Busserolles J, Deval E, Alloui A, Diochot S, et al. The
 854 mechano-activated K⁺ channels TRAAK and TREK-1 control both warm and cold
 855 perception. *EMBO J.* 2009;28:1308-18.

(157) Papka RE, Hafemeister J, Storey-Workley M. P2X receptors in the rat uterine cervix, lumbosacral dorsal root ganglia, and spinal cord during pregnancy. *Cell Tissue Res.* 2005;321:35-44.

(158) Ikeuchi M, Kolker SJ, Burnes LA, Walder RY, Sluka KA. Role of ASIC3 in the primary and secondary hyperalgesia produced by joint inflammation in mice. *Pain.* 2008;137:662-9.

(159) Verge GM, Milligan ED, Maier SF, Watkins LR, Naeve GS, Foster AC. Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. *Eur J Neurosci.* 2004;20:1150-60.

(160) Zhao Q, Liang X, Han H. [Cannabinoid receptor 1 controls nerve growth in ectopic cyst in a rat endometriosis model]. *Zhonghua Bing Li Xue Za Zhi.* 2014;43:827-30.

(161) Zador F, Wollemann M. Receptome: Interactions between three pain-related receptors or the "Triumvirate" of cannabinoid, opioid and TRPV1 receptors. *Pharmacol Res.* 2015;102:254-63.

Table 1. Different types of peripheral nerve fibers.

Type of fibers		Function	Axonal diameter (μm)	Conduction velocity (m/s)	Direction of conduction
A (myelinated)	α	Proprioception, muscle contraction	12-22	70-120	Afferent and efferent
	β	Touch-pressure, vibration	6-12	80	Afferent and efferent
	γ	Intrafusal fibers contraction	4-8	15-30	Efferent
	δ	Pain (acute, shallow), temperature (cold receptors), touch-pressure	1-5	4-30	Afferent
B (myelinated)		Preganglionic fibers of ANS	1-3	3-15	Efferent
C (unmyelinated)	Fibers of dorsal roots	Pain (chronic, deep), temperature (warmth receptors), touch-pressure (tactile or mechanoreceptors)	0.2-1.5	0.5-2	Afferent
		Postganglionic fibers of sympathetic nerves	0.2-1.5	0.5-2	Efferent

ANS: autonomic nervous system. Compiled from references [22, 23].

881

882

Table 2. Markers used to identify nerve fibers in endometriosis of different types.

Molecules	Nerve fibers detected	Function	Eutopic Endometrium			Ectopic Endometrium				Adenomyosis	Associated with pain	References
			Functional Layer	Basal Layer	Myometrium	DIE	Peritoneal Lesions	Ovarian Lesions	Intestinal Lesions			
PGP9.5	Pan-neuronal marker (myelinated and unmyelinated fibers)	Highly specific to neurons and cells of the diffuse neuroendocrine system and their tumors	↑	↑	↑	↑ (higher than any other ectopic lesions and eutopic endometrium)	↑	↑	—	↓	✓	[34, 35, 38, 39, 44-52]
S100	Pan-neuronal marker (myelinated fibers)	A family of calcium-binding proteins, regulating inflammatory cells, neurons, astrocytes, microglia, and endothelial and epithelial cells	↑	—	↑	↑	—	—	—	○	✓	[28, 53-56]
NF	Pan-neuronal marker (myelinated fibers)	A major component of the neuronal cytoskeleton, providing structural support for the axon	↑	●	↑	↑	↑	↑	—	↓	✓	[35, 38, 39, 45, 46, 48-50]
NSE	Pan-neuronal marker	Used to identify neuronal cells and a marker for neuroendocrine tumors	↑	—	—	↑ (non-significant)	—	—	—	—	✓	[56, 57]
SP	Sensory nerves (Aδ and C fibers)	Involved in pain generation, immunomodulation, neurite outgrowth, angiogenesis and proliferation of fibroblasts	↑	●	○	↑	↑	↑	○	↓	✓	[34, 35, 38, 39, 46, 47, 52, 56, 58, 60, 61]
CGRP	Sensory nerves (Aδ and C fibers)	Involved in vasodilatation, transmission of pain, immunomodulation, angiogenesis	↑	●	○	↑	↑	—	—	—	✓	[35, 38, 39, 46, 47, 52]
NPY	Sympathetic nerves (adrenergic fibers); Sensory nerves (Aδ and C fibers)	Involved in vasoconstriction, reducing anxiety and stress, also reducing pain perception	↑	●	○	↑	—	↑	—	—	—	[35, 38, 39, 46, 47, 56-59]
TH	Sympathetic nerves (adrenergic fibers)	The rate-limiting enzyme of catecholamine biosynthesis, present in the CNS, peripheral sympathetic neurons and adrenal medulla	×	●	×	↑	↓	—	↓	↓	—	[35, 38, 39, 46, 52, 60, 61]
VMAT	Sympathetic nerves (monoaminergic fibers)	A transport protein integrated into the membrane of synaptic vesicles of presynaptic neurons, acting to transport monoamine neurotransmitters	—	—	↑	—	—	—	—	—	✓	[34]
VIP	Parasympathetic nerves (cholinergic fibers); Sensory nerves (Aδ and C fibers)	Involved in vasodilatation, anti-inflammatory, stimulating NGF and VEGF production	↑	●	—	↑	—	↑	—	—	✓	[35, 38, 39, 47, 56-58]
ACh	Parasympathetic nerves (cholinergic fibers); Sensory nerves (Aδ and C fibers)	A neurotransmitter released by motor neurons, also as the final product released by the parasympathetic nervous system	×	×	○	○	—	—	—	—	—	[39, 46]
GAP-43	Regenerated nerve fibers	A crucial component of the axon and presynaptic terminal, distinguishing newly grown nerve fibers from existing nerves	↑	—	—	—	—	—	—	—	✓	[62, 63]

883

884 ● Present in women with endometriosis only

885 ↑ Increased density in women with the diseases

886 ↓ Decreased density in women with the diseases

887 ○ No differences between women with and without endometriosis

888 × Not detected

889 — Not reported

890 ✓ Reported

891

892

893

894

895

896

Table 3. Published studies on the use of nerve fiber density in endometrium for diagnostic purpose.

	Markers	# of cases	# of controls	Samples	Specificity	Sensitivity	Accuracy	Positive predictive value (PPV)	Negative predictive value (NPV)	References
	PGP9.5 and NF	10	35	Functional and basal layer of endometrium	100%	100%	NA	100%	100%	[38, 39]
Positive evidence	PGP9.5	20	17	Functional layer of endometrium	100%	100%	NA	100%	100%	[138]
	PGP9.5	64	35	Functional layer of endometrium	83%	98%	NA	91%	96%	[37]
	Combination of PGP9.5, VIP and SP	20	20	Functional layer of endometrium in women with endometriosis	100%	95%	97.5%	100%	95%	[47]
	PGP9.5	12	15	Functional layer of endometrium	80%	100%	96.1% (ROC curve)	NA	NA	[139]
	PGP9.5	10	10	Eutopic endometrium	50%	100%	NA	NA	NA	[140]
	Combination of PGP9.5 and serum IL-6	74	40	Functional layer of endometrium in women with endometriosis	92.5%	100%	NA	100%	92.7%	[141]
	PGP9.5	30	30	Eutopic endometrium	100%	80%	NA	NA	NA	[142]
	PGP9.5	16	13	Endometrium in women with endometriosis	100%	81.3%	NA	NA	NA	[143]
	S100	16	15	Endometrium in women with pain	40%	81.3%	NA	NA	NA	[54]
Negative evidence	PGP9.5	77	13	Functional layer of endometrium in women with pain	NA	83.3%	NA	NA	NA	[48]
	PGP9.5	29	37	Functional layer of endometrium	75.7%	37.9%	NA	NA	NA	[49]
	PGP9.5	47	21	Functional layer of endometrium	71.4%	19.1%	NA	NA	NA	[67]
	PGP9.5, p75 and TRPV1	20	25	Functional layer of endometrium	NA	NA	NA	PGP9.5: P=0.0991, TRPV1: P=0.0621	NA	[144]
	PGP9.5 and NF	31	29	Functional layer of endometrium	0%	0%	NA	NA	NA	[145]
	PGP9.5 and NF	26	20	Functional and basal layer of endometrium	0%	0%	NA	NA	NA	[146]
	PGP9.5	22	22	Functional layer of endometrium	Endometrial curettings: 45.5% Endometrial biopsies: 68.2%	31.8% 13.6%	NA NA	36.8% 30.0%	40.0% 44.1%	[147]

PGP9.5: protein gene product 9.5; VIP: vasoactive intestinal peptide; SP: substance P;
S100: S100 protein; IL: interleukin; TRPV1: transient receptor potential vanilloid 1;
NF: neurofilament; NA: data not available.

Table 4. List of possible perpetrators suspected to be involved in the generation of endometriosis-associated pain.

Roles	Candidates	Ligands	Related function	References
Promotional	TXA2	-	Expressed in rat cortical neurons, and promotes neurite outgrowth via MAPKs	[88]
	PGE2	-	A direct generators of pain, triggering the release of other algescic mediators (NGF, histamine, serotonin, prostanoids)	[6]
	NGF	TrkA, p75, TRPV1, NMDARs	Contained in PF from women with endometriosis, and the NGF blockade significantly reduced neurite outgrowth	[81, 116, 148]
	BDNF	TrkB, p75, NMDARs	Expressed in endometriotic lesions, triggering activation of NMDARs in sensory terminals during neuropathic pain	[83, 108, 149]
	SP	NK1R	Except for hyperalgesia and central sensitization, the expression of NK1R may permit endometriotic lesion maintenance via exposure to SP	[135, 149, 150]
	ADRB2	Epinephrine, NE	Adrenergic stimulation results in increased PGE2 synthesis via ADRB2, driving proliferation and angiogenesis	[133, 151]
	TRPV1	Noxious heat (>43°C), tissue acidosis	Estrogen-dependent increase of TRPV1-positive nerve fibers in endometriosis may integrate various stimuli on sensory terminals and generate hyperalgesia	[92, 93, 95]
	Na _v 1.7, Na _v 1.8, Na _v 1.9	-	Primarily expressed in nociceptors, with also a higher expression in EAP, as key relay stations for the electrical transmission of pain signals from the periphery to CNS	[95, 101, 117, 152]
	MCP-1	CCR2	Increased in endometriosis, may excite primary sensory and DRG neurons by upregulating the current density and expression of TRPV1 and Na _v 1.8	[153, 154]
	KCNK2 (TREK-1), KCNK4 (TRAAK)	Noxious heat (ca 40°C) and cold, pressure	Regulating nociceptors activation of sensory neurons by temperature and mechanical stimuli	[117, 155, 156]
	KCNK18 (TRESK)	Hydroxy- α -sanshool	Primary contributor to the excitatory effects of sanshool in sensory neurons, and depolarizes the nociceptors	[117, 118]
	NMDARs	Glutamate, BDNF, NGF	Expressed both pre- and post-synaptically in nociceptive neurons, increasing neurotransmitter release such as SP	[108, 109, 149]
	P2XR3	ATP	Initiating nociceptive signals interpretable as pain, and elevated in the peritoneal tissue of women with CPP	[95, 157]
	ASIC3	Protons (tissue acidosis)	Located in primary afferent fibers, ASIC3 is critical for the development of inflammatory hyperalgesia	[158]
Inhibitory	CX3CR1	Fractalkine (CX3CL1)	Mediating communication between DRG neurons and microglia associated with nociceptive transmission, also expressed in endometriotic lesions, increasing the migration and adhesion of neuronal cells in pancreatic cancer	[129, 131, 159]
	mGluR2/3	Glutamate	Modulating neurotransmission all along the pain neuraxis, and blocking TRPV1-induced activation of nociceptors	[113, 114]
	CB1R	Endocannabinoid	Interacted with opioid and TRPV1 receptors, CB1R expressed on both the sensory and sympathetic neurons that innervate endometriosis's abnormal growths and migration	[115, 160, 161]

ADRB2: β 2 adrenergic receptor; ASIC3: the acid sensing ion channel 3; BDNF:

1 910 brain-derived neurotrophic factor; CB1R: cannabinoid receptor 1; CCR2: C-C
2
3 911 chemokine receptor type 2; CNS: central nervous system; CPP: chronic pelvic pain;
4
5
6 912 CX3CR1: CX3C chemokine receptor 1; DRG: dorsal root ganglion; EAP:
7
8
9 913 endometriosis-associated pain; KCNK: potassium channel subfamily K member;
10
11
12 914 MCP-1: monocyte chemotactic protein 1, also known as chemokine ligand 2 (CCL2);
13
14
15 915 mGluR2/3: group II/III of the metabotropic glutamate receptors; Na_v1.7, Na_v1.8,
16
17 916 Na_v1.9: sodium voltage-gated ion channels; NE: norepinephrine; NK1R: neurokinin
18
19
20 917 receptor 1; NMDAR: N-methyl-D-aspartic acid receptor; PGE₂: prostaglandin E2;
21
22
23 918 P2XR3: P2X purinoceptor 3; SP: substance P; TRPV1: transient receptor potential
24
25
26 919 cation channel subfamily V member 1; TXA₂: thromboxane A2.
27
28 920
29
30
31 921
32
33
34 922
35
36 923
37 924
38 925
39
40 926
41 927
42 928
43
44 929
45 930
46 931
47
48 932
49 933
50
51 934
52 935
53 936
54
55 937
56 938
57
58 939
59 940
60
61
62
63
64
65

Table 5. Neuromediators involved in different phases of wound healing. Summarized from [22, 120].

Wound Healing	Associated neuromediators
Inflammatory phase	SP, CGRP and NPY induce the release of CKs Bradykinin, 5-HT, PGs, CKs stimulate nociceptors present on C-nerve fibers TRPV1 sensitization causes the depolarization of nociceptors and transduction of pain
Proliferative phase	CGRP and VIP may be involved in the re-epithelialization by keratinocyte proliferation; NGF acts on not only mast cells but also B and T lymphocytes to facilitate proliferation and differentiation of fibroblasts, promoting fibroblast migration in the granulation tissues
Remodeling phase	SP, CGRP and VIP modulate MMP-2 and MMP-9 activities, and are involved in the conversion of collagen III to collagen I

CKs: cytokines; CGRP: calcitonin gene-related peptide; MMP: matrix metalloproteinase; NGF: nerve growth factor; NPY: nerve peptide Y; PGs: prostaglandins; SP: substance P; TRPV1: transient receptor potential vanilloid 1; VIP: vasoactive intestinal peptide.

Figure legend

Figure 1. Classifications of nerve fibers and their relationship with eutopic and ectopic endometrium. Symbols used in the figure:

- Efferent fibers; — Afferent fibers;
- Increased density in women with endometriosis;
- Decreased density in women with endometriosis
- Present in women with endometriosis only
- No differences between women with and without endometriosis

Abbreviations used: ACh: acetylcholine; CNS: central nervous system; DA: dopamine; DRG: dorsal root ganglion; E: epinephrine; NE: norepinephrine; PNS: peripheral nervous system.

Figure 2. Schematic illustration of possible cross-talks between endometriotic lesions and nerve fibers. Abbreviations used in the figure: ACh: acetylcholine;

ADRB2: β 2 adrenergic receptor; ASIC3: the acid sensing ion channel 3; BDNF: brain-derived neurotrophic factor; CGRP: calcitonin gene-related peptide; CX3CR1: CX3C chemokine receptor 1; GAP43: growth-associated protein 43; GDNF: glial-derived neurotrophic factor; KCNK: potassium channel subfamily K member; Na_v1.8: sodium voltage-gated ion channels; NE: norepinephrine; NGF: nerve growth factor; NK1R: neurokinin receptor 1; NMDAR: N-methyl-D-aspartic acid receptor; NT-3: neurotrophin-3; NT-4/5: neurotrophin-4/5; PGE₂: prostaglandin E2; P2XR3: P2X purinoceptor 3; SP: substance P; TRPV1: transient receptor potential cation channel subfamily V member 1; TXA₂: thromboxane A2; VEGF: vascular endothelial growth factor. The question mark indicates the role of indicated molecule in endometriosis-associated pain, if any, is yet to be investigated.

Figure 1.

Possible cross-talks between endometriotic lesions and nerve fibers