Eutopic endometrium and peritoneal, ovarian and colorectal endometriotic tissues express a different profile of Nectin-1, -3, -4 and nectin-like molecule 2

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STUDY QUESTION: How is the expression of nectins and nectin-like molecules (Necls) detected by immunostaining altered by endometriosis?

SUMMARY ANSWER: Our results suggest that Nectin-1, -3, -4 and Necl-2 may contribute to the pathogenesis of endometriosis. Immunostaining of nectins and Necls varies according to the anatomical location of endometriosis.

WHAT IS KNOWN AND WHAT THIS PAPER ADDS: Nectin and Necl molecules are immunoglobulin-like cell adhesion molecules involved in apoptosis, cell proliferation and in metastases. Previous studies have demonstrated the involvement of adhesion molecules in the development of endometriotic lesions but no data exist on immunostaining of nectins and Necls molecules in endometriosis.

DESIGN, PARTICIPANTS AND SETTING: This retrospective study was conducted in a tertiary-care hospital (Tenon Hospital, Paris, France). Samples were collected from 55 women undergoing endometrial biopsy or surgery for endometriosis and 20 controls having hysterectomy or endometrial biopsy for other reasons; multiple samples were collected from 15 women. We studied the immunostaining of Nectin-1, -3, -4 and Necl-2 in secretory and proliferative endometrium from women with (n = 20) or without endometriosis (i.e. control group, n = 20), and in peritoneal (n = 20), ovarian (n = 20) and colorectal endometriosis (n = 20).

MAIN RESULTS: Semi-quantitative immunostaining demonstrated that (1) Necl-2 staining was stronger in all types of endometriotic lesions than in the eutopic endometrium from patients with endometriosis (P < 0.0125) and in ovarian endometriotic cysts compared with other locations (P < 0.001); (2) Nectin-3 staining was stronger in the eutopic endometrium of patients with endometriosis compared with controls (P = 0.03) and in all endometriotic lesions compared with the eutopic endometrium from patients with endometriosis (P < 0.0125); (3) Nectin-4, staining was stronger in the eutopic endometrium of patients with endometriosis compared with controls (P = 0.04) and (4) Nectin-1 staining was significantly increased in colorectal endometriosis compared with other locations (P = 0.004).

BIAS, CONFOUNDING AND OTHER REASONS FOR CAUTION: We did not assess the pattern of expression in endometriosis of all nectins and Necl molecules. Indeed, Necl-5 is implicated in many pathophysiological processes such as cell movement and proliferation with potential relevance to endometriosis.

GENERALISABILITY TO OTHER POPULATIONS: At present, few data on implication of nectins and Necl molecules in endometriosis exist. Hence, our results should be confirmed by further quantitative studies at protein or RNA levels.

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Key words: endometriosis / immunohistochemistry / nectin molecules / nectin-like molecules
Introduction

Endometriosis is a benign disorder causing pain and infertility characterized by the presence of endometrial glands and stroma outside the uterus (Dubernard et al., 2008; de Ziegler et al., 2010). Though often associated, there are three typical locations of endometriosis: peritoneal, ovarian (also called endometrioma) and deep infiltrating endometriosis (Somigliana et al., 2004). The development of endometriosis depends on estrogen levels and the induction of angiogenesis and lymphangiogenesis (Nisolle et al., 2007). Moreover, ectopic implantation of endometrial cells is a result of complex interactions between host tissue and epithelial endometrial cells (Nisolle et al., 2007). Previous studies have demonstrated the involvement of adhesion molecules in the development of endometriotic lesions. One of these showed that loss of E-cadherin expression is associated with invasiveness of endometriotic lesions (Poncelet et al., 2002). Another study demonstrated that over-expression of some matrix metalloproteinases (MMP-2 and -3) is related to the infiltration of the retroperitoneal space with increased expression in colorectal endometriosis compared with peritoneal endometriosis (Uzan et al., 2004).

Nectins and nectin-like molecules (Necls) have been recently identified as immunoglobulin-like cell adhesion molecules (CAMs) regulating the formation of cell–cell junctions (adherens junctions) and the formation of tight junctions in epithelial cells (Takai et al., 2008). They also participate in the regulation of cellular activities such as cell polarization, differentiation, movement, proliferation and survival (Takai and Nakanishi, 2003; Takai et al., 2003). Furthermore, several studies have shown the involvement of nectins and Necls in various pathways of malignant tumor development, including Nectin-3 in apoptosis, Nectin-4 in the occurrence of metastases and Necl-2 in cell proliferation (Takai et al., 2008). Nectin-1 has also been shown to play an important role in neurogenesis such as axon guidance (Okabe et al., 2004). Moreover, small nerve fibers, which are responsible for endometriosis-related pain, also play an important role in the development of endometriotic nodules (Anaf et al., 2004, 2011). Hence, these pathways have been previously implicated in the occurrence of endometriotic lesions (Uzan et al., 2004; Nisolle et al., 2007). However, the involvement of nectins and Necls in the pathogenesis of endometriosis has never been studied before.

Therefore, the aims of this study were to examine Nectin-1, Nectin-3, Nectin-4 and Necl-2 expression in the eutopic endometrium from women with or without endometriosis, and to compare their expression in peritoneal, ovarian and colorectal endometriotic tissues.

Materials and Methods

Patients

The study was conducted in the Department of Obstetrics and Gynecology of Tenon Hospital, Paris. Tissue samples consisted of 20 endometrial tissues, 20 ovarian endometriotic cysts, 20 peritoneal endometriotic tissues and 20 colorectal endometriotic tissues from 75 premenopausal women requiring surgical treatment. Several tissue samples were collected from the same patient in 15 cases (women with endometriosis). In addition, normal endometrial samples were obtained from 20 women undergoing hysterectomy or endometrial biopsy for benign diseases. No macroscopic endometriotic features were found during laparoscopy or laparotomy for patients included in this group.

In the group of women without endometriosis, samples were obtained during the proliferative phase of the menstrual cycle in 14 cases and during the secretory phase in 6. In the group of women with endometriosis, samples were obtained in the proliferative phase in 15 cases and in the secretory phase in 5. The mean ages of patients according to the type of tissue collected were: endometriotic lesions 36 years (range 26–48), eutopic endometrium from women without endometriosis 43.7 years (range 39–49) and eutopic endometrium from women with endometriosis 39 years (range 30–48). None of the patients had received hormone therapy for at least 3 months before surgery. All the tissue samples were obtained with full and informed patient consent.

Statistical analysis

The $\chi^2$ test was used for comparisons of qualitative immunostaining. The Kruskal–Wallace and Mann–Whitney tests were used for comparisons of semi-quantitative immunostaining. $P$-values of $<0.05$ denoted a significant difference. To control for multiple comparisons (four at each time point),
Expression of nectins in endometriosis

Results

Qualitative and semi-quantitative immunostaining of nectins and Necl-2 in the endometrium

Immunostaining was restricted to the membrane in glandular cells for all the nectins and Necl-2 (Fig. 1). No difference in Nectin-1 (P = 1), Nectin-3 (P = 0.16), Nectin-4 (P = 0.23) or Necl-2 (P = 1) expression was observed according to the phase of the menstrual cycle (i.e. proliferative and secretory phases).

The results of the qualitative immunostaining of Nectin-1, -3, -4 and Necl-2 are summarized in Table I. No difference in qualitative immunostaining was found in Nectin-1, -3, -4 or Necl-2 between the endometrium from patients with or without endometriosis. The semi-quantitative immunostaining of Nectin-1, -3, -4 and Necl-2 in the endometrial samples using HSCORE analysis is shown in Fig. 2. A higher immunostaining of Nectin-3 and Nectin-4 was found in the eutopic endometrium from patients with endometriosis (P = 0.03 and P = 0.048, respectively). No significant difference in the immunostaining of Nectin-1 or Necl-2 was found between endometrial samples.

Qualitative and semi-quantitative immunostaining of Nectin-1, -3, -4 and Necl-2 in the eutopic endometrium from women with endometriosis and in peritoneal, ovarian and colorectal endometriosis

Nectin-2 immunostaining was more common in peritoneal endometriosis (90%, P = 0.01), ovarian endometriosis (100%, P = 0.001) and colorectal endometriosis (80%, P = 0.02) compared with the eutopic endometrium from women with endometriosis (50%). Nectin-1 immunostaining was more common in colorectal endometriosis (100%, P = 0.01) compared with the endometrium from women with endometriosis (40%). No difference was found for Nectin-3 or -4 (Table II).

Using HSCORE analysis, semi-quantitative Nectin-3 and Necl-2 immunostaining was found to differ depending on the location of endometriosis (Fig. 3). Nectin-3 immunostaining was higher in peritoneal endometriosis (HSCORE = 230 ± 80; P = 0.0002), ovarian endometriosis (HSCORE = 230 ± 70; P = 0.0002) and colorectal endometriosis (HSCORE = 250 ± 80; P < 0.0001) compared with the eutopic endometrium from women in this group (HSCORE = 150 ± 80). Nectin-2 immunostaining was higher in peritoneal endometriosis (HSCORE = 190 ± 100; P < 0.0001), ovarian endometriosis (HSCORE = 260 ± 60; P < 0.0001) and colorectal endometriosis (HSCORE = 100 ± 70; P = 0.002) compared with the endometrium from women with endometriosis (HSCORE = 30 ± 40). No difference was found for Nectin-1 or -4.

Qualitative and semi-quantitative immunostaining of nectins and Necl-2 in peritoneal, ovarian and colorectal endometriosis

The results of the qualitative immunostaining of Nectin-1, -3, -4 and Necl-2 according to the location of the endometriotic lesions are summarized in Table II. Nectin-1 immunostaining was found in 100% of colorectal endometriosis samples, 50% of ovarian endometriotic cysts and 30% of peritoneal endometriosis samples. Nectin-1 immunostaining was more common in colorectal endometriosis than in peritoneal and ovarian endometriosis (P = 0.004). No differences were observed for Nectin-3, -4 or Necl-2.

Using HSCORE analysis, semi-quantitative Nectin-1 and Necl-2 immunostaining was found to differ according to the location of endometriosis (Fig. 3). Nectin-1 immunostaining was higher in colorectal endometriosis (HSCORE = 100 ± 30) than in peritoneal endometriosis (HSCORE = 60 ± 70) and ovarian endometriotic cysts (HSCORE = 16 ± 38) (P = 0.005). Necl-2 immunostaining was higher in ovarian endometriotic cysts (HSCORE = 260 ± 60) than in peritoneal (HSCORE = 190 ± 100) and colorectal endometriosis (HSCORE = 100 ± 70) (P < 0.0001). No differences were observed for Nectin-3 or -4.

Discussion

Several differences in the qualitative and semi-quantitative expression of Nectin-1, -3, -4 and Necl-2 were observed in the endometrium of patients with endometriosis and in peritoneal, ovarian and colorectal endometriosis.

Our results demonstrate an increased expression of Necl-2 in the ectopic endometrium. We found a higher qualitative and semi-quantitative expression of Necl-2 in all types of endometriotic lesions compared with the eutopic endometrium of patients with endometriosis. Moreover, differences were also found between ectopic lesions: higher semi-quantitative expression was found in ovarian endometriotic cysts compared with peritoneal and colorectal lesions. Necl-2 has been identified as a tumor suppressor leading to the arrest of cell proliferation and apoptosis, with low or loss of expression in tumor cells (Mao et al., 2004; Fournier et al., 2010). It is frequently inactivated in lung, pancreas and liver carcinomas (Kuramochi et al., 2001; Fournier et al., 2010) and several authors have demonstrated that the tumor suppression mechanism involves interaction with the Rac and Akt signaling pathways (Kawano et al., 2009; Ogita et al., 2010). While Necl-2 participates in the inhibition of Rac and Akt pathways in normal epithelial cells, these pathways are activated following the reduction or loss of expression of Necl-2 in tumor cells facilitating cell proliferation and metastases (Ogita et al., 2010). This could explain why low expression of Necl-2 in colorectal endometriosis compared with peritoneal endometriosis and ovarian endometriotic cysts results in the high potential for invasion of the retroperitoneal space. This hypothesis is supported by Plyoshi and Takai (2007) who have shown that reducing the activity of Necl-2 can lead to disruption of cell polarity and adhesion, resulting in a neoplastic growth.

We found that Nectin-3 also contributes to the pathogenesis of endometriosis with an increased expression in the endometrium we applied a Bonferroni correction, setting the significance level at 0.002: P < 0.0125 was considered to denote statistical significance.
Figure 1 Representative examples of membrane Nectin-3 immunostaining (A) in endometrium. Nectin-3 immunostaining in the endometrium from women without (B) and with (C) endometriosis. Nectin-1 immunostaining in the peritoneal (D) and colorectal (E) endometriosis. Necl-2 immunostaining in colorectal (F) and ovarian (G) endometriosis. Immunoperoxidase staining with dianminobenzidine as chromogen, and nuclear counterstaining with Harris hematoxylin.
of patients with endometriosis compared with the endometrium of those without. In addition, it appears that the semi-quantitative expression of Nectin-3 is higher in all endometriotic lesions compared with the endometrium of patients with endometriosis. Nectin-3 plays a crucial role in apoptosis through its interaction with platelet-derived growth factor (PDGF). Several studies have shown significant physical and functional interactions between molecules of the CAM family and growth factor receptors (Hayama et al., 2006; Kato et al., 2007; Takano et al., 2009). This interaction could inhibit apoptosis and contribute to cell survival induced by PDGF. Moreover, afadin, which interacts directly with nectin molecules, also has an anti-apoptotic activity through the PI3K-AKT signaling pathway (Takano et al., 2009). This anti-apoptotic activity has been demonstrated by previous studies showing a decrease in apoptosis in the endometrium of patients with endometriosis compared with the endometrium of those without endometriosis, and a higher expression of Bcl-2 (Dufournet et al., 2006). Our results are consistent with these findings: we found higher Nectin-3 expression in the endometrium from women with endometriosis compared with the endometrium from women without endometriosis.

Many reports have demonstrated physical and functional associations between CAMs and cadherins, integrins and PDGF in regulating contact inhibition of cell movement and proliferation (Takai et al., 2008). Moreover, nectins (especially Nectin-3) physically associate with integrin αvβ3 at cell–cell adhesion sites, an association that is essential for the nectin-induced activation (Takai et al., 2008). In a study comparing mRNA expression of adhesion factors in women with and without endometriosis, Kyama et al. (2008) showed that during the menstrual phase, mRNA endometrial expression of αvβ3 integrin was higher in patients with endometriosis. The same authors suggested that αvβ3 integrin may facilitate the adhesion of endometrial cells to the pelvic peritoneum, especially during menstruation, leading to the development of endometriosis. Hence, this is in total accordance with our results, which show an increased expression of Nectin-3 in the endometrium of patients with endometriosis.

Our results also show a higher semi-quantitative expression of Nectin-4 in the endometrium of patients with endometriosis.
compared with the endometrium of those without. Several studies have shown an increased expression of Nectin-4 in ovarian cancer and a correlation between the expression of Nectin-4 and the occurrence of metastases in breast and lung cancers (Fabre-Lafay et al., 2005, 2007; Takano et al., 2009). The main pathophysiological mechanism that has been put forward is that, in contact with a suitable site, the Nectin-4 on the surface of ectopic or metastatic cells undergo cleavage leading to increased intercellular adhesion and invasion of

**Table II** Qualitative expression of Nectin-1, -3, -4 and Necl-2 in peritoneal endometriosis, ovarian endometriotic cysts and colorectal endometriosis and in the eutopic endometrium from women with endometriosis.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Samples with positive staining/n (%)</th>
<th>Eutopic endometrium</th>
<th>Peritoneal endometriosis</th>
<th>Ovarian endometriotic cysts</th>
<th>Colorectal endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nectin-1</td>
<td>8/20 (40)</td>
<td>10/20 (50)</td>
<td>6/20 (30)</td>
<td>20/20 (100)*</td>
<td></td>
</tr>
<tr>
<td>Nectin-3</td>
<td>17/17 (100)</td>
<td>20/20 (100)</td>
<td>20/20 (100)</td>
<td>20/20 (100)</td>
<td></td>
</tr>
<tr>
<td>Nectin-4</td>
<td>16/20 (80)</td>
<td>18/20 (90)*</td>
<td>15/20 (75)</td>
<td>19/20 (95)</td>
<td></td>
</tr>
<tr>
<td>Necl-2</td>
<td>10/20 (50)</td>
<td>18/20 (90)*</td>
<td>20/20 (100)*</td>
<td>16/20 (80)</td>
<td></td>
</tr>
</tbody>
</table>

*Eutopic endometrium was compared with peritoneal, ovarian and colorectal endometriosis using the χ² test; we applied a Bonferroni correction: \( P < 0.0125 \) was considered to denote statistical significance.

**Figure 3** Comparison of semi-quantitative expression of Nectin-1, -3, -4 and Necl-2 between ovarian endometriotic cysts, peritoneal endometriosis and colorectal endometriosis (\( P \)-value noted *; Kruskal–Wallis test). Comparison of semi-quantitative expression of Nectin-1, -3, -4 and Necl-2 between ovarian endometriotic cysts, peritoneal endometriosis, colorectal endometriosis and the eutopic endometrium from women with endometriosis (\( P \), 0.0125 noted ** was considered to denote statistical significance; Mann–Whitney test after Bonferroni correction); NS, not significant.
the site. This hypothesis could explain the metastatic potential of endometrial cells. Moreover, the invasive capacity of ectopic endometriotic cells has been reported in previous studies showing a loss of expression of E-cadherin (Darai et al., 1998; Uzan et al., 2004). Finally, the invasion phase also involves the MMPs and their natural inhibitors and tissue inhibitor metalloproteinases (TIMPs). Many studies have found overexpression of MMPs and a lower concentration of TIMPs in the eutopic and ectopic endometrium as well as in the peritoneal fluid in patients with endometriosis compared with healthy subjects (Uzan et al., 2004). It seems that Nectin-4 may participate in the mechanisms of invasion of subperitoneal space by ectopic endometrial cells in a way similar to E-cadherin and MMPs.

In contrast, no data on the involvement of Nectin-1 in the physiopathology of endometriosis are available although it has been extensively studied in the pathway of the herpes virus. A previous study has shown a trans-interaction between Nectin-1 and -3 (Narita et al., 2011). It has also been shown to organize the heterotypic cell–cell adhesion and regulate changes in the trajectory of commissural axons (Okabe et al., 2004). Several markers of neurogenesis, such as Pgp 9.5, have recently been found in the endometrium from symptomatic patients with endometriosis compared with patients without (Tokushige et al., 2007). Moreover, Pgp 9.5 expression in the endometrium has been proposed as a new diagnostic tool for endometriosis. Several studies have also demonstrated that, in addition to the inflammatory response to the ectopic endometrium, there is a higher number of nerve structures in endometriotic nodules contributing to the occurrence of severe and neuropathic pain that characterizes these lesions (Anaf et al., 2004, 2011). Myelinated and unmyelinated nerve fibers in direct contact with peritoneal endometriotic lesions, as well as the de novo innervation of peritoneal lesions, in a rodent endometriosis model have been demonstrated (Berkley et al., 2004). There is evidence that endometriosis may be related to neurotrophic events because neurotrophins, such as the nerve growth factor and neurotrophin 3, are expressed in peritoneal endometriotic implants (Barcena de Arellano et al., 2011). Finally, it is important to note that colorectal endometriosis representing the most aggressive form of endometriosis is characterized by both an increased expression of Nectin-1 and decreased expression of Necl-2.

In conclusion, this preliminary study suggests the contribution of Nectin-1, -3, -4 and Necl-2 to the pathogenesis of endometriosis. Expression of nectins and Necls varies according to the anatomical localization of endometriosis, as has been demonstrated for other adhesion molecules such as cadherins and MMPs, confirming the complexity of the development of endometriotic lesions.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org/.

**Authors’ roles**

All the authors took part in the design and implementation of the study, and read and approved the final report. The corresponding author has had access to all the data in this study and he had final responsibility for the decision to submit for publication. The first author, M.B. from the Department of Obstetric and Gynecology, ‘Hôpital Tenon, Assistance Publique des Hôpitaux de Paris, Paris, France’, collected and analyzed all the data, performed the statistical analysis and wrote the article. J.G. collected the data from patients, analyzed all the data and drafted the article. A.R. collected the data from patients and analyzed all the data. J.-F.B. analyzed the data and drafted the article. R.R. analyzed all the data and approved the final version of the article. C.C. analyzed all the data, performed the statistical analysis and wrote the article. E.D. collected the data from patients, performed the statistical analysis and wrote the article.

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**Conflict of interest**

None declared.

**References**


