L1 cell adhesion molecule (L1CAM) as a pathogenetic factor in endometriosis

D. Finas1,†, M. Huszar2,†, A. Agic1, S. Dogan1, H. Kiegel3, S. Riedle3, D. Gast3, R. Marcovich2, F. Noack4, P. Altevogt3, M. Fogel2 and D. Hornung1,5

1Department of Obstetrics and Gynecology, University of Schleswig-Holstein, Ratzeburgerallee 160, 23538 Luebeck, Germany; 2Department of Pathology, Kaplan Medical Center, 76100 Rehovot, Israel; 3Tumorimmunology Programme, D010, German Cancer Research Center, 69120 Heidelberg, Germany; 4Department of Pathology, University of Schleswig-Holstein, 23538 Luebeck, Germany

†Both authors contributed equally to this work.

BACKGROUND: Endometriosis is a benign and progressive disease with a high prevalence. Women with endometriosis, especially with atypical endometriosis, have a higher probability for developing ovarian cancer compared with women without endometriosis. The L1 cell adhesion molecule (L1CAM) is over expressed in ovarian and endometrial carcinomas and is associated with a bad prognosis. Here, we have analysed L1CAM expression in endometriosis.

METHODS AND RESULTS: In our study with the samples from 79 patients with, and 37 patients without, endometriosis, we found that endometriosis cell lines and short-term cultures of endometrium from women with endometriosis expressed L1CAM at the mRNA and protein level. Quantitative RT-PCR analysis showed that L1CAM was expressed at significantly higher level in the epithelial compartment from patients with endometriosis compared with healthy controls (P = 0.0126). By immunohistochemical staining, 15 of 31 ovarian endometriotic lesions (48%) were shown to have L1CAM-positive staining. Of these 15 L1CAM-positive samples, 13 were atypical endometriotic lesions. Soluble L1 present in the conditioned medium of epithelial endometrium cultures from women with endometriosis was able to stimulate neurite outgrowth as measured in a chicken ganglion assay.

CONCLUSIONS: We propose that L1CAM could promote endometriosis development by increasing enervation and aggravation. L1CAM expression is higher in atypical endometriosis compared with normal endometriosis.

Keywords: atypical endometriosis; endometriosis; immunohistochemistry; L1CAM; quantitative RT-PCR

Introduction

Endometriosis is a benign and most frequently progressive disease with a high prevalence in the reproductive-phase of the female population. Overall, studies estimate that endometriosis may affect around 7–15% of women of reproductive age, thus making this a common condition. Nevertheless, endometriosis shows behavior similar to malignant tumors and deep invasion in different tissues such as peritoneum, ovary and intestines is caused by migrating epithelial and stromal cells of modified endometrium, adhering to distant tissues (Witz et al., 2003). Endometriosis is associated with an increased risk for several types of malignant diseases (Sherman et al., 1992; Magtibay et al., 2001; Berglund et al., 2003; Blumenfeld, 2004). In particular, the endometrioid subtype of ovarian cancer has a high probability for developing on the basis of existing endometriosis (Ridley, 1966; Czernobilsky et al., 1970a,b; Czernobilsky and Morris, 1979).

Like malignancy, endometriosis displays features of atypia, adherence, invasion and metastases. Atypical endometriosis is characterized histologically by endometrial glands with cytological or architectural atypia (LaGrenade and Silberberg, 1988), and has been observed in 12–35% of ovarian endometriosis (Seidmann, 1996; Nishida et al., 2000; Bayramoglu and Duzcan, 2001). Around 60–80% of cases of endometriosis-associated ovarian cancer (EAOC) occur in the presence of atypical ovarian endometriosis (Fukunaga et al., 1997; Ogawa et al., 2000; Oral et al., 2003). Of these cases, 25% show direct continuity of the atypical ovarian endometriosis with ovarian cancer, underlying a potential ‘premalignant’ transition spectrum of non-atypical to atypical endometriosis and malignant variants.

Around 60% of EAOCs occur with the cancer adjacent to endometriosis or arising directly from ovarian endometriosis, with the remaining 40% occurring with distant endometriotic disease (Modesitt et al., 2002). Clear-cell and endometrioid carcinomas are the most common EAOCs with ovarian endometriosis, whereas clear-cell adenocarcinoma and...
adenosarcoma are the commonest EAOCs in extra-ovarian endometriosis (Stern et al., 2001; Zaino et al., 2001). The risk of direct malignant transformation of ovarian endometriosis has been estimated as 0.7–1.6% over an average of 8 years.

L1 cell adhesion molecule (L1CAM, CD171) is a type I transmembrane glycoprotein (200–220 kD), which belongs to the immunoglobulin superfamily (Rathjen and Schachner, 1984). Initial studies have shown that L1CAM plays an important role in the development of the nervous system and is involved in several morphogenic events, such as neuron–neuron adhesion, neurite fasciulation, synaptogenesis, neurite outgrowth on Schwann cells and neuronal cell migration (Montgomery et al., 1996; Schachner, 1997; Hortsch, 2000). More recent studies have implicated L1CAM also in the ontogeny of human tumors including melanomas, neural tumors, renal carcinomas, colon carcinoma and endometrial and ovarian carcinomas (Fogel et al., 2003; Huszar et al., 2006; Kaifi et al., 2006; Meier et al., 2006). The expression of L1CAM in carcinomas augments dissemination of tumor cells by enabling cell migration and invasion (Montgomery et al., 1996; Felding-Habermann et al., 1997; Ohnishi et al., 1998; Mechtersheimer et al., 2001) and promotes epithelial–mesenchymal transition (Shutman et al., 2006). Given the role of L1 in endometrial and ovarian carcinomas, we investigated in the present report the expression of L1CAM in endometriosis.

Materials and Methods

Patient recruitment and source of tissue

Endometrial biopsies were collected by a pipelle, ovarian endometriotic tissue was collected by laparoscopy and abdominal wall scar endometriosis was collected by laparotomy. All tissue types were confirmed by histology. Tissue, serum and peritoneal fluid (PF) samples were obtained from patients undergoing laparoscopy after providing written consent under a study protocol approved by the University of Luebeck, Germany and by the Kaplan Hospital Rehovot, Israel committee on human research. All recruited patients were without any endocrine or anti-inflammatory therapy for at least 6 months before surgery. Patients in the control group (Co) underwent laparoscopy because of uterine myomata or were without pelvic pathology and requesting tubal ligation without any clinical or pathological evidence of endometriosis. The biopsies for cell culture experiments (only typical ovarian endometriosis cysts and endometrium from women with and without endometriosis) and serum and PF samples were taken in the midproliferative-phase of the menstrual cycle under sterile conditions (38 patients with endometriosis and 19 patients without endometriosis contributed to cell culture experiments). For immunohistochemistry, 18 normal endometrium samples (10 proliferative- and 8 secretory-phase) and 41 endometriosis samples (31 ovarian cysts: 17 typical endometriosis and 14 atypical endometriosis, and 10 abdominal wall scar endometriosis: 6 typical endometriosis and 4 atypical endometriosis) were used from a tissue collection archive (Kaplan Hospital, Rehovot, Israel). Atypical endometriosis is defined as reactive atypia and atypical mitoses in glandular epithelium; stromal changes including smooth muscle metaplasia and decidualization; lipoblast-like cells, some with intranuclear inclusions; atypical or degenerative myocytes and spiral arteries. Ultrastructurally, these cells show cytoplasmatic granules, some with a delimiting membrane. All atypical endometriosis samples showed p53 over expression.

The endometriosis group (Eo) consisted of 79 patients and the Co of 37 patients (age: 36.7 ± 6.5 and 33.3 ± 8.5 years (mean ± SD, n.s.), who underwent surgery at the Department of Obstetrics and Gynecology, University of Luebeck, Germany and the Kaplan Hospital Rehovot, Israel. Patients with endometriosis were staged intraoperatively according to the revised American Fertility Society-Score (rAFS-Score) in Stages I–IV (AFS, 1997).

Cell isolation, purification and culture

Cell cultures were prepared from endometrium. Endometrial tissue was dissected free from underlying myometrium or parenchyma and minced into small pieces, digested with collagenase (2 mg/ml) for 1 h at 37°C, and separated using serial filtration. Debris was removed by 100 μm aperture sieves, and epithelial glands were retained on 40 μm aperture sieves, and backwashed onto tissue culture dishes. Stromal cells remaining in the filtrate were plated onto Primaria flasks (Becton Dickinson, Lincoln Park, NJ, USA) and allowed to adhere for 30 min, after which blood cells were removed with phosphate-buffered saline rinses. The cells were cultured in RPMI medium (Cambrex, Verviers, Belgium) reconstituted with 10% FCS, nucleosides, essential amino acids, penicillin G (100 U/ml), streptomycin (100 μg/ml) and fungizone (1 μg/ml). The FCS contained 22.5 ng/ml estradiol, which results in a final concentration of 2.25 ng/ml estradiol in the cell culture medium. Stromal cultures were dissociated with 0.05% trypsin and 0.02% versene in saline, harvested by centrifugation, replated and allowed to grow to confluence. Purification of the stromal cell population was confirmed by negative staining for CD3 (T cells), CD11b (granulocytes), CD45 (monocytes and other leucocytes) and cytokeratin (epithelial cells) and positive staining for vimentin. Purification of the epithelial cells was confirmed by cytokeratin positive and vimentin negative staining. Cell viability was confirmed by the CellTiter-Blue® Cell Viability Assay (Promega, Mannheim, Germany). The epithelial endometriosis derived cell lines Z12 and Z49 were a kind gift of Prof. Starzinski-Powitz (University of Frankfurt, Germany). Cells were cultivated as described (Zeitvogel et al., 2001) and were used between passages 15 and 17.

Chicken ganglion assay

Valo SPF oocytes (Lohmann Tierzucht, Cuxhaven, Germany) were incubated for 12 days. The embryos were taken out with a pincet on the 12th day after start of incubation and decapitated in a Petri dish with cold PBS. The spinal cord was prepared under a microscope and the ganglions were collected. Ganglions were divided in 8-well dishes, shortly incubated with Collagen G (Biochrom, Berlin, Germany) and washed. The ganglions were incubated at 37°C and 5% CO2 with different media: (i) RPMI (negative control), (ii) RPMI+ NGF (nerve growth factor, 10 ng/ml, positive control), (iii) endometrium epithelial cell culture conditioned media from women with endometriosis (without and with different concentrations of L1-antibody, 32–325 μg/ml) and (iv) the identical endometrium epithelial cell culture conditioned media from women with endometriosis with L1 depleted conditioned media (conditioned media after preincubation with sepharose-coupled L1-mAb to remove soluble L1). After 48 h, the ganglions were fixed, stained with neurofilament-antibodies immunofluorescence and photographed. The growth of the nerve fibers was measured and described on a scale from 0 to 3 (0 = no growth, 1 = scant growth, less than 1/3 of ganglion size, 2 = intermediate growth, from 1/3 up to 2/3 of ganglion size, 3 = dense growth, more than 2/3 of ganglion size).
**Immunohistochemistry**

The immunohistochemical methods have been described (Fogel et al., 2003). Briefly, serial 4 µm tissue sections from formalin-fixed, paraffin embedded, endometriotic lesions and endometrium were placed on SuperFrost Plus slides (Menzel-Glaeser, Braunschweig, Germany). After incubating with primary antibody L1-11A (subclone of UJ127.11 (Arlt et al., 2006)) overnight at 4°C, sections were incubated with biotinylated goat anti-mouse IgG (Vectorstain Elite, Vector Laboratory, Burlingame, CA, USA) for 30 min, then for another 30 min with Avidin–Biotin–Peroxidase complex (Vectorstain Elite). Subsequently, slides were incubated in peroxidase substrate solution (AEC, Zymed Laboratory, San Francisco, CA, USA). After rinsing with water, sections were counterstained with Hematoxylin (Merck, Darmstadt, Germany), dehydrated and covered with glycerin gelatin (Merck). The presence of L1CAM-positive peripheral nerves served as an internal positive control.

**Extraction and purification of mRNA from tissues and cells**

Total mRNA was extracted from whole tissues (endometrial and endometriotic) and from cell cultures (endometrial and endometriosis cell lines) using the RNA Easy Kit according to the manufacturer’s protocol (Qiagen, Hilden, Germany).

**Real-time reverse transcription-PCR**

Polyadenylated mRNA was preferentially reverse transcribed using oligo(deoxythymidine) primers. Specific oligonucleotide primers were designed to amplify sequences from human L1CAM, and ADAM10 mRNA. The primers used for RT-PCR experiments are shown in Table I. The PCR conditions were as described previously (Fogel et al., 2003). Briefly, PCR cycling was preceded by an initial denaturation step at 95°C for 1 min, followed by one annealing step for over 1 min at 56°C, and then 33 cycles with annealing and extension at 72°C. The PCR products were separated on 2% agarose gels (3% NuSieve GTG, 1% SeaKem GTG, FMC Bioproducts, Rockland, ME) and visualized by ethidium bromide staining (Life Technologies, Gaithersburg, MD). Hypoxanthine–guanine phosphoribosyl transferase (HPRT) was used as a control gene in real-time PCR for all samples.

**Western blot analysis**

The western blot analysis has been described in detail (Itoh et al., 2000). Briefly, cell lysates from cultured endometrial stromal and epithelial cells from women with endometriosis or endometriosis cell lines were subjected to SDS–gel, transferred to nitrocellulose membranes or PVDF membranes, and blocked with 5% fat dry milk prior to incubations with mAb L1-11A. Bound antibody was detected with peroxidase-conjugated goat anti-mouse IgG and ECL detection.

**Statistical analysis**

Differences between the Co and the Eo were evaluated using the non-parametric Mann–Whitney-U-Test, one-way ANOVA in combination with Tukey’s Multiple Comparison and t-test and results were considered statistically significant when two tailed analyses yielded P < 0.05. All statistical analyses were performed with Prism 4 for Windows, GraphPad Software, 2003, San Diego, USA.

### Results

**Detection of L1CAM in endometriosis cell lines**

Since it is not possible to culture endometriotic epithelial primary cells in a sufficient number for FACS analyses, western blot or quantitative real-time PCR, we used two human endometriosis epithelial cell lines for these initial experiments. A recent publication has shown that cell lines with a limited life span can be established from endometriotic lesions (Zeitvogel et al., 2001). We examined two of these cell lines, Z12 and Z49 for expression of L1CAM. The Z12 cells were clearly positive for L1CAM expression as detected by FACS (Fig. 1A) and western blot analysis (Fig. 1B). In contrast, Z49 cells showed a much weaker reactivity. Both cell lines expressed L1CAM-specific mRNA as detected by RT-PCR (Fig. 1C).

**Analysis of L1CAM in short-term cultures of endometrium from patients with and without endometriosis**

We extended our analysis to short-term cultures of endometrium obtained from patients with and without endometriosis. The epithelial and stromal components were separated as described in Materials and Methods and the cells were cultured

### Table I. Primers for L1CAM, ADAM10, and HPRT used in PCR analysis.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1CAM forward</td>
<td>gca gca agg gcc aat act ca</td>
</tr>
<tr>
<td>L1CAM reverse</td>
<td>ctt gat gcc ccc gtt gag cga t</td>
</tr>
<tr>
<td>ADAM10 forward</td>
<td>cag aat gac caa gag aa</td>
</tr>
<tr>
<td>ADAM10 reverse</td>
<td>gtt ttc ccc cag aat tgg ct</td>
</tr>
<tr>
<td>Exon 27 forward</td>
<td>act cag tga aga ata agg ag</td>
</tr>
<tr>
<td>Exon 27 reverse</td>
<td>tgg agc gat ggc tgg ctg ct</td>
</tr>
<tr>
<td>HPRT forward</td>
<td>cct ggc gtc gtt atg gat</td>
</tr>
<tr>
<td>HPRT reverse</td>
<td>cca gca gtt cag caa aga att ta</td>
</tr>
</tbody>
</table>

Figure 1: Expression of L1CAM in endometriosis epithelial cell lines.

(A) FACS analysis of Z12 and Z49 endometriosis cell lines. Cells were analysed by cytfluorographic analysis using mAb L1-11A to L1CAM followed by Phycoerythrin-conjugated anti-mouse IgG antibody. (B) Western blot analysis on cell lysates from Z12 and Z49 cells. Blots were probed with mAb L1-11A followed by peroxidase-conjugated goat anti-mouse IgG and ECL detection. (C) Real-time RT-PCR using L1-specific primer. Z12 values were set to 1 in order to allow a comparison of expression levels.
for 7 days before mRNA isolation. RT-PCR analysis showed that L1CAM was significantly more highly expressed in the epithelial compartment of the endometrial cell culture from patients with endometriosis Stages I to IV (57.7 ± 13.5) compared with endometrial epithelium from healthy controls (13.2 ± 6.3, \( P = 0.0126 \)) (Fig. 2A). There was no statistical difference in L1CAM expression between the endometrial stromal cells of endometriosis patients and controls (data not shown). In a subanalysis, patients with myomata compared with women without pelvic pathology requesting tubal ligation (both in the Co) did not show any difference in L1CAM expression. The difference in L1CAM expression in endometrial epithelial cells was especially high between patients with endometriosis rAFS Stages III and IV (55.7 ± 15.5) compared with endometrial epithelium from healthy controls (13.2 ± 7.3, \( P = 0.0076 \)) (Fig. 2B). Patients in the endometriosis (36.5 ± 7.0) and Co (34.3 ± 6.3) were age matched (\( P > 0.05 \)). Western blot analysis on cell lysates from epithelial and stromal cultures from patients with endometriosis confirmed the presence of L1CAM at the protein level in epithelial cells (Fig. 2C). To exclude the possibility that the L1CAM expression in our in vitro endometrial cells in an artifact, we performed microdissection and examined the expression of L1CAM mRNA in epithelial and stromal cells. The results were similar to those obtained by stromal and epithelial cells after cell culture (data not shown). The amount of tissue after microdissection, however, was not sufficient for western blot experiments. Therefore, we have shown only the results after cell culture experiments.

**L1CAM in short-term cultures of endometrium from women with endometriosis is devoid of exon 27**

L1CAM can be expressed in differentially spliced forms. The neural form of L1 includes the two exons 2 and 27, respectively, that are missing in epithelial and hematopoietic cells (Itoh et al., 2000). Since endometrium of women with endometriosis is often well enervated and peripheral nerve cells could give rise to cellular contamination in short-term cultures, we analysed the expression of exon 27 in epithelial cultures by RT-PCR using a specific primer. As shown in Fig. 3, L1CAM forms in short-term culture were devoid of exon 27 expression ruling out the possibility of cellular contamination with neural cells. In none of the analysed cDNA samples from endometriosis patients (\( n = 40 \)), the expression of exon 27 was observed (Fig. 3).

**Analysis of ADAM10 in short-term cultures of endometrium from women with and without endometriosis**

L1CAM can be released in a soluble form by ectodomain shedding involving the metalloproteinases ADAM10 (Felding-Habermann et al., 1997; Beer et al., 1999; Gutwein et al., 2000, 2003; Kyama et al., 2003). We therefore examined the expression of ADAM10. The RT-measurement of ADAM10 mRNA from endometrial cell cultures from endometriosis patients (epithelium: 118.4 ± 30.6, stroma: 124.0 ± 26.8) and controls (epithelium: 58.1 ± 24.2, stroma: 47.9 ± 8.5) showed statistically significant differences within the epithelial and stromal compartment of patients with endometriosis in comparison to that in healthy controls (\( P = 0.042 \)) (Fig. 4).

**Figure 2:** Detection of L1CAM in short-term cultures of endometrium from patients with and without endometriosis. (A) RT-PCR for L1CAM from endometrium cell culture (epithelium) of patients with (Eo), and without (Co), endometriosis. (B) RT-PCR for L1CAM from endometrium cell culture (epithelium) of the patients with endometriosis AFS stages III and IV and controls. (C) Western blot for L1CAM from endometrium cell culture (epithelium and stroma) from patients with endometriosis. Cultures from three representative patients are shown.

**Figure 4:** ADAM10 expression in short-term cultures of endometrium from patients with and without endometriosis. Real-time PCR for ADAM10 from endometrium cell culture (epithelium and stroma) of patients with (Eo) and without (Co) endometriosis...
Detection of L1CAM in endometrium and endometriosis by immunohistochemical staining

Immunohistochemical staining of endometriotic samples showed high expression of L1CAM adhesion molecule in the epithelial sections. Representative staining examples are depicted in Fig. 5. L1CAM was absent from proliferation- (Fig. 5A) or secretory-phase (Fig. 5B) endometrial epithelial cells from healthy controls. In typical endometriosis epithelial samples, 88.2% were L1 negative (Fig. 5C) and 11.8% were L1 positive (Fig. 5D). In contrast, L1CAM was especially highly expressed in the epithelium of atypical endometriotic lesions (Fig. 5E). On the other hand, the stromal compartment showed very little if any L1CAM staining intensity. As expected, the internal control, peripheral nerves, were positive for L1CAM staining (not shown). As summarized in Table II, from 31 samples of ovarian endometriotic lesions, 15 (48.4%) showed L1CAM-positive immunohistochemical staining. Of these 15 L1CAM-positive samples, 13 (58.8%) were atypical endometriotic lesions. Of 13 L1CAM-negative samples, only 1 (7.7%) was an atypical endometriotic lesion. Endometriotic lesions from abdominal wall showed a similar distribution: of

<table>
<thead>
<tr>
<th>Site/Histology</th>
<th>No. of total cases</th>
<th>No. of L1-positive cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>31</td>
<td>2 (11.8)</td>
</tr>
<tr>
<td>Typical</td>
<td>17</td>
<td>13 (92.9)</td>
</tr>
<tr>
<td>Atypical</td>
<td>10</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Abdominal wall</td>
<td>6</td>
<td>4 (100)</td>
</tr>
</tbody>
</table>

Figure 5: Immunohistochemical staining of endometrium and endometriosis lesions
(A and B) L1CAM expression is absent in proliferative or secretory endometrium from controls. (C) A representative case of L1 negative typical ovarian endometriosis. (D) A representative case of L1-positive typical ovarian endometriosis (inset: magnification ×40). (E) A case of L1-positive ovarian atypical endometriosis (inset: magnification ×40). All stainings were carried out on paraffin-embedded tissues using mAb L1-14.10. (magnification ×20)
10 samples, 5 (50%) were L1CAM positive. Of the five L1CAM positive samples, four (80%) were atypical endometriotic lesions and of the five L1CAM-negative lesions, only one (20%) was an atypical endometriotic lesion (Table II).

**PF and serum L1CAM ELISA**

Soluble L1CAM is present in serum and ascites fluid of patients with ovarian carcinoma tumors (Fogel et al., 2003). We therefore examined whether soluble L1CAM could be detected in the PF of patients with and without endometriosis using a sensitive L1-specific ELISA (Mechtersheimer et al., 2001). We observed that soluble L1CAM levels in the PF of patients (n = 21) with endometriosis (0.80 ± 0.26 ng/ml, P > 0.05) did not significantly differ from the levels in healthy controls (n = 11, 0.99 ± 0.36 ng/ml).

**Effect of culture supernatant from endometrial epithelial cultures of women with endometriosis on neurite outgrowth**

Endometriotic lesions are often well enervated and can cause considerable pain to affected women. Soluble L1 is known as a potent stimulator of neurite outgrowth (Hartwig et al., 2006). We argued that although soluble L1 could not be detected systemically in body fluids, it could well act locally when released from the lesion to promote neurite growth. In order to test this hypothesis, we used the chicken ganglion assay to study nerve growth-promoting activity in the conditioned medium of endometrial epithelial cell cultures from women with endometriosis. As shown in Fig. 6A, the unstimulated chicken ganglions showed no growth of neurites after 48 h incubation with cell culture medium alone (negative control), whereas after stimulation with neurite growth factor (NGF 7S, 10 ng/ml) for 48 h, obvious neurite growth was detectable (positive control, Fig. 6B). The conditioned medium of endometrium epithelial cell cultures from patients with endometriosis stimulated similar strong neurite growth compared with the positive control in this assay (Fig. 6C). We were able to block this supernatant-dependent growth by depletion, the supernatant showed a significant although not complete inhibition of neurite outgrowth suggesting that beside soluble L1, other growth-promoting factors are present in the conditioned medium of endometriosis cultures (Fig. 6D). The data on neurite outgrowth are summarized in Fig. 6G.

**Discussion**

Endometriosis and malignant disorders show some characteristic similarities, such as dissemination, deep invasion, expansive behavior, increased angiogenesis, high rate of recurrence with chronic course and progression. A severe epithelial atypism in endometriotic spots may mimic a premalignant lesion. Carcinomas could occur on the grounds of such cell atypism (Czernobilsky and Morris, 1979) and endometrioid histological types of ovarian cancers arise in 15% of cases from ovarian endometriomas (Ridley, 1966; Czernobilsky et al., 1970a,b). Cancerous development in endometriotic tissue, which is known to proliferate under estradiol stimulation, is possible even years after deprivation of the estrous stimulus by ovariectomy (Magtibay et al., 2001). Berglund et al. reported in a study on Swedish women with endometriosis, that 3418 malignomas occur in 753 838 women years. The tumor incidence was not different to that in the normal population. But the risk for certain malignomas such as ovarian cancer, Non-Hodgkin-lymphomas, tumors with endocrine activity and several brain tumors was significantly augmented (Berglund et al., 2003).

Dissemination and adhesion of endometriotic tissues depend on multiple factors. Changes in the expression of cell adhesion molecules (CAMs) could be involved in these processes (Moos et al., 1988; Witz, 2003). Recently, one of these molecules, the neural cell adhesion molecule L1CAM (CD171), was detected in ovarian and uterine carcinomas. It was first described as being involved in neural cell adhesion and as a stimulator of neurite outgrowth (Lindner et al., 1983; Rathjen and Rutishauser, 1984). Lindner et al. (1983) showed the role of L1CAM in building central nervous structures by specific blocking of neurite cell migration using mono- and polyclonal L1CAM antibodies. Additionally, L1CAM is involved in neurite outgrowth in the peripheral nervous system (Chang et al., 1990). Lack of L1CAM leads to severe deletions in the whole neuronal setting, such as non-myelination and reduced axonal sprouting (Dahme et al., 1997). Serious pelvic pain, such as dysmenorrhea and dyspareunia, is a frequent symptom of endometriosis. Endometriosis-associated pelvic pain may be promoted by L1CAM induced neurite outgrowth in the endometriotic tissue. It is possibly mediated by ADAM10 related cleavage of the L1CAM ectodomain resulting in increased nerve outgrowth. Soluble L1CAM is found in the serum and ascites of uterine and ovarian carcinoma patients and high levels were found to be a bad prognostic factor (Fogel et al., 2003). But in contrast to the findings in malignant disorders, soluble L1CAM was not increased in serum samples of patients with endometriosis. Nevertheless, ADAM10 was found in the epithelial cell fraction of endometrium derived from patients with endometriosis which could explain the presence of soluble L1CAM in the supernatant of short-term epithelial cultures from women with endometriosis. In a rat model of surgically induced endometriosis, Berkley et al. found that ectopic endometrial growth induced vaginal hyperalgesia as well as autonomic and sensory innervations (Cason et al., 2003; Berkley et al., 2001, 2004, 2005).

The present study shows that L1CAM is present in the endometriotic tissue of women with endometriosis. L1CAM expression was studied by RT-PCR at the message and at the protein level using short-term cultures of endometrium epithelial cells from women with endometriosis. Since we could not rule out the possibility that the in vitro culture
conditions could influence the expression of L1CAM as in the case of cultured human keratinocytes (Meier et al., 2006), we used immunohistochemical staining of endometrial lesions to verify our results. About 50% of the endometrial lesions showed L1CAM-positive staining. From these 15 L1CAM-positive samples, 13 were atypical endometriotic lesions. The expression of L1CAM increases from typical endometriosis (11.8% L1CAM-positive samples) to atypical endometriosis (92.9% L1CAM-positive samples, own results) and ovarian cancer (79.3% positive samples in: Fogel et al., 2003).

We speculate that L1CAM may be associated with increased nerve growth and pain in endometriosis. Recently, Schwarz et al. showed that sensory nerve fibers are located in the stroma of peritoneal endometriotic lesions. They suggest that these nerve fibers could play an important role in the etiology of pain in endometriosis. Using a neural growth assay, they could demonstrate a neurotropic stimulating effect of ectopic endometrial glands and stroma to the growth of nerve fibers even into endometriotic lesions (Schwarz et al., 2005).

Figure 6: Stimulation of neurite outgrowth by conditioned medium of endometrium epithelial cells from women with endometriosis. (A) Unstimulated chicken ganglions showed no growth of neurites after 48 h incubation with cell culture medium, negative control. (B) Stimulation of chicken ganglions with neurite growth factor (NGF 7S, 10 ng/ml) for 48 h, positive control. (C) Stimulation with conditioned medium of endometrium epithelial cell cultures from patients with endometriosis. (D) Stimulation with the same conditioned medium in the presence of 32 μg/ml or (E) 325 μg/ml of L1-mAb L1-11A. (F) Stimulation with the same conditioned medium depleted for soluble L1 by sepharose-L1-11A (coupled at 1 mg/ml) (magnification ×100). (G) A quantification of the results measuring the length of 15 nerves per ganglion per group.
Finas et al.

The expression of L1CAM in malignant disorders occurs in a stage-dependent manner and is correlated with tumor progression and metastasis (Fogel et al., 2003) by enabling migration, adhesion, invasion and proliferation of tumor cells (Montgomery et al., 1996; Felding-Habermann et al., 1997; Beer et al., 1999). Proteolytic cleavage and release of the extracellular domain of L1CAM involving proteinases like ADAM10 occurs in several malignant disorders (Beer et al., 1999; Gutwein et al., 2000, 2003). Cell migration and survival through autocrine and/or paracrine binding to integrins can be stimulated by soluble L1CAM (Beer et al., 1999; Voura et al., 2001), but also through direct L1CAM to cell-interaction (Beer et al., 1999; Fogel et al., 2003). Shed L1CAM can serve as a substrate for adhesion and migration of tumor cells (Duczmal et al., 1997).

On the basis of the previous reflections, we hypothesize that L1CAM could promote endometriosis development and aggravation. We found a significantly higher expression of L1CAM in the epithelial cell fraction in rAFS Stages III and IV (AFS 1997) endometriosis compared to controls. Endometriotic tissue is formed as a unit of epithelium and stromal components (Ryan et al., 1994). As yet it is unclear whether it implants by interaction of only one of these components or both. We propose that the dissemination and adhesion of endometriotic tissue in endometriosis could be promoted by L1CAM.

An important characteristic of endometriotic lesions is the high potential of neovascularization (McLaren, 2000). Extensive blood supplies both within and surrounding the ectopic tissue is essential for its survival. Pancook et al. (1997) showed significant L1CAM expression in endothelial cells associated with the arterioles and red pulp of normal spleen. Additionally, a Dutch group found a L1CAM over expression in endothelial cells of blood vessels of deep infiltrating endometriotic lesions of the rectovaginal space (Van Langendonckt et al., 2005). They isolated the cells by laser capture microdissection and measured L1CAM expression using microarray analysis validated by real-time RT-PCR. ADAM10 mediated L1CAM cleavage triggers L1CAM haptotactic cell migration (Gutwein et al., 2003). Neovascularization and vessel sprouting may be induced through ADAM10 mediated L1CAM shedding into the extracellular matrix (ECM) (Montgomery et al., 1996; Gutwein et al., 2003) surrounding endometriotic implants. The L1CAM–L1CAM or L1CAM–integrin interaction between the ECM and endothelial blood cells could support the attachment of endothelial cells as the beginning of new vessel outgrowth (Gutwein et al., 2000).

The accumulation of immune cells, especially activated macrophages, is thought to mediate endometriosis related inflammatory symptoms (Haney et al., 1981; Olive et al., 1985). Cells of myelomonocytic and lymphoid origin in the human immune system show L1CAM expression (Kowitz et al., 1992; Ebeling et al., 1996) and L1CAM may be involved in migration of activated T-lymphocytes (Ebeling et al., 1996). L1CAM is expressed on activated macrophages (Duczmal et al., 1997) which are, in addition to activated T-lymphocytes, of particular importance in mediating inflammatory symptoms associated with endometriosis (Hornung et al., 2001). The cell adhesion molecule L1CAM may contribute to cell–cell adhesion events associated with extravasation of those immune effector cells (Ebeling et al., 1996).

Anti-L1CAM-cell adhesion molecule monoclonal antibody treatment was successfully able to inhibit the intra-peritoneal tumor growth and dissemination of ovarian carcinoma cells in nude mice. A dose-dependent and significant reduction of tumor burden (up to −63.5%) and ascites formation (up to −75%) was reported (Arlt et al., 2006). Future studies are needed to demonstrate, if this antibody can also be helpful for the treatment of endometriosis.

In summary, L1CAM is mainly expressed in the epithelial compartment of endometrium and endometriosis. The expression of L1CAM is increased in atypical endometriosis and ovarian cancer compared with typical endometriosis. We speculate that L1CAM may be associated with increased nerve growth and pain in endometriosis.

Acknowledgements

We wish to thank Prof. Starzinski-Powitz (University of Frankfurt, Germany) for the endometriosis cell lines Z12 and Z49. Special thanks also goes to Gabriele Marschner and Constanze Siggel for expert technical help.

Funding

M.F. and P.A. were supported from the European Union by EC-STREP OVCAD. P.A. was supported by the Deutsche Krebshilfe, Schwerpunktprogramm ‘Invasion and Metastasis’. 

References


Submitted on May 6, 2007; resubmitted on January 19, 2008; accepted on January 30, 2008