The Differential Role of L1 in Ovarian Carcinoma and Normal Ovarian Surface Epithelium

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Abstract
Epithelial ovarian carcinoma (EOC) arises from the ovarian surface epithelium (OSE), a monolayer of poorly differentiated epithelial cells that lines the ovary. The molecular mechanisms underlying EOC invasion into the surrounding stroma and dissemination to the peritoneum and to retroperitoneal lymph nodes are still unclear. Here, we analyzed the expression and the functional role of the cell adhesion molecule L1 during EOC development. In patient-derived samples, L1 was expressed both in OSE and in a subset of EOC, in the latter being mostly restricted to the invasive areas of the tumors. The expression of L1 correlated significantly with poor outcome and with unfavorable clinicopathologic features of the disease. The peculiar expression pattern of L1 in normal OSE and invasive EOC raised the possibility that this adhesion molecule serves a different function in nontransformed versus neoplastic ovarian epithelial cells. Indeed, we showed that in OSE cells L1 supports cell-cell adhesion and enhances apoptosis, whereas it has no effect on cell proliferation and invasion. In contrast, L1 inhibits cell-cell adhesion and apoptosis in ovarian carcinoma cells, where it promotes malignancy-related properties, such as cell proliferation, Erk1/2-dependent and phosphoinositide 3-kinase–dependent invasion, and transendothelial migration. Interestingly, a crosstalk with the fibroblast growth factor receptor signaling is implicated in the promalignant function of L1 in tumor cells. Our findings point to L1 as an EOC biomarker correlating with poor prognosis, and highlight a switch in L1 function associated to the neoplastic transformation of ovarian epithelial cells, thus implicating L1 as a potential therapeutic target. [Cancer Res 2008;68(4):1110–8]

Introduction
Epithelial ovarian carcinoma (EOC) is the leading cause of mortality among gynecologic malignancies in the Western world. Due to the lack of clinically relevant symptoms during the early phases of cancer development, the diagnosis is frequently made at advanced stages of the disease. Surgical cytoreduction and chemotherapy usually induce tumor regression; however, most patients undergo a relapse of the disease with a high mortality rate (1). Although a number of genetic lesions associated to higher risk for specific forms of EOC have been identified, only very few biomarkers that contribute to the early diagnosis and prognosis of the disease are currently available, accounting for the difficult management of this tumor type. Moreover, the molecular mechanisms that control the onset and progression of EOC have remained elusive, and their definition would certainly open novel therapeutic avenues for the treatment of such a life-threatening disease.

Cell adhesion molecules (CAM) are cell surface proteins mediating cell-cell and cell-matrix interactions. Alterations in CAM expression and/or function have been implicated in the development of various tumor types. In particular, the dysregulation of cell-cell adhesion caused by changes in the levels of cadherins and/or immunoglobulin-like CAMs (Ig-CAM) plays a causal role in the progression of several epithelial tumors (2). The Ig-CAM L1 (also known as L1CAM or CD171) has been extensively characterized in the nervous system, where it mediates neuronal adhesion and migration, as well as axon pathfinding and fasciculation (3). However, L1 is also expressed in nonneuronal tissues, including certain epithelia and some hematopoietic lineages, where its function remains elusive. Recent studies have reported an aberrant expression of L1 in various tumor types, including melanoma (4) and colon carcinoma (5). In the latter, L1 has been characterized as a transcriptional target of the Wnt/β-catenin pathway and as a factor involved in tumor cell invasion (5). Finally, an aberrant expression of L1 has been described in advanced EOC and has been proposed to enhance the malignant phenotype of EOC cells (6, 7). Here, we report the first demonstration that the expression of L1 is not restricted to EOC but is also abundant in the ovarian surface epithelium (OSE), the monolayer of poorly differentiated epithelial cells lining the ovaries that, upon neoplastic transformation, gives rise to most EOC forms (8). Furthermore, in agreement with previous reports, our analysis of clinical specimens revealed that the expression of L1 correlates with poor prognosis in EOC patients. The expression pattern of L1 and its association with specific clinicopathologic features of the disease suggested that this adhesion molecule serves a different function in normal versus neoplastic OSE. To verify this hypothesis, we have characterized the biological activities of L1 in OSE and in EOC cell lines. Our results indicated that L1 plays markedly different, and often opposite, roles in nontumorigenic versus transformed ovarian epithelial cells. These findings underscore the importance of the cellular context in dictating the functional role of L1 in OSE cells and provide the rationale...
for evaluating L1 both as a EOC biomarker and as a novel target for the molecular therapy of ovarian carcinoma.

Materials and Methods

Selection of the patients, tissue microarray analysis, and data analysis. Clinicopathologic and follow-up data of ovarian cancer patients surgically treated at the European Institute of Oncology from 1995 to 2004 were used to select the cases that were included in the study. Inclusion criteria were (a) first surgery at the European Institute of Oncology, (b) no neoadjuvant treatment, and (c) common epithelial histologic subtypes (serous, mucinous, and endometrioid cystoadenocarcinomas).

For clinicopathologic details of the patients, construction of tissue microarrays, and data analysis, see Supplementary Materials and Methods.

Cell culture. Human ovarian carcinoma cell lines IGROV1 and OVCAR3 were from American Type Culture Collection.

Methods for details on selection and analysis of patients). The expression of L1 was investigated in a panel of surgically resected specimens, including 20 normal ovaries with a clearly detectable OSE, 4 benign lesions (cystadenoma), 211 primary EOC, and 199 metastatic lesions (see Supplementary Materials and Methods for details on selection and analysis of patients). The results are summarized in Fig. 1C and Supplementary Table S2. The immunohistochemical staining with the polyclonal antibody pcatL1, directed against the cytoplasmic tail (11), showed high levels of L1 in 100% of the normal OSE samples, mainly localized at the intercellular boundaries and at the basolateral surface of the cells (Fig. 1A, left). The identity of L1-positive tissue as OSE was confirmed by the positive staining of the same cells for cytokeratins (Supplementary Fig. S1C–E). By immunohistochemistry, benign lesions showed an L1 immunoreactivity similar to OSE (not shown). Because our observations were in contrast with a previous study that reported the absence of L1 in normal OSE (6), we used additional antibodies (2C2, CE7, and L1-S; see Supplementary Table S1) that confirmed the L1 immunoreactivity in OSE tissue (Supplementary Fig. S1A and data not shown). Furthermore, L1 was also detected in freshly isolated OSE cells where, by analogy to normal ovary, it was enriched at cell-cell contacts of primary OSE cells (Fig. 1A, right and Supplementary Fig. S1B). The immunoblotting of OSE cell lysates showed that L1 migrated as a doublet of 200 to 220 kDa (Supplementary Fig. S3), where the higher molecular weight band is likely to represent the mature, surface-exposed protein, whereas the lower molecular weight form would represent the intracellular pool of L1 (12).

Nine primary EOC (43%) and 89 metastases (45%) exhibited a clear L1 staining (Fig. 1C). However, in contrast to nonneoplastic epithelium (where 100% of OSE cells exhibited L1 staining), the vast majority of cancer cells within these tumor tissues showed no or very little expression of L1 in the tissue cores analyzed [primary EOC, mean 7.89% (0–80); metastatic cancer, mean 7.74 (0–100)]. The immunoreactivity of L1, instead, was specifically maintained in less cohesive cells inside the tumor mass and at the invasive front of the tumor (Fig. 1B and Supplementary Fig S2B and C). Because this pattern suggested that L1 could be implicated in loss of cell-cell adhesion and in cancer invasion (and, hence, progression), we asked whether the expression of L1 in tumor specimens was associated with the clinicopathologic features of the disease. Indeed, the presence of L1 in primary EOC significantly correlated with the poorly differentiated, more aggressive phenotype of the tumor cells (grade 3, P = 0.034; see Supplementary Table S2). In addition, tumors characterized by advanced International Federation of Gynecology and Obstetrics stage (III–IV versus I–II) and by the presence of lymph node metastasis exhibited higher L1 levels, although the correlation did not reach statistical significance (P = 0.064 and 0.057, respectively). Kaplan-Meier survival analyses strengthened the prognostic value of L1 by showing a significantly shorter 5-year overall (log rank, P = 0.0087) and disease-free (log rank, P = 0.029) survival in patients with L1-positive tumors (Fig. 1D), thus confirming and extending the results of a previous study performed on a lower number of cases (6). To determine if L1 expression was a prognostic factor independent from the other clinicopathologic variables, we performed a multivariate proportional hazards modeling of overall and disease-free survival, including patient's age, histologic grade, and tumor stage. Importantly, the presence of L1 was independently associated with shorter 5-year overall survival (Fig. 1D, left), whereas the correlation with shorter disease-free survival, despite showing a clear trend, did not reach statistical significance (Fig. 1D, right).

In summary, our screening of human ovarian samples revealed that (a) L1 expression is significantly down-regulated in primary and metastatic EOC compared with normal OSE and (b) the expression of L1 in ovarian cancer cells correlated with clinicopathologic features of aggressiveness and with poor outcome. These apparently contradictory results, together with the frequently observed L1 immunoreactivity in less cohesive cells within the tumor mass and at the invasive front of EOC, suggest that a switch in L1 function might occur upon transformation of ovarian epithelial cells.

To address the role of neoplastic transformation in L1 expression, we took advantage of a genetically defined cellular model that recapitulates many features of EOC development. This consisted of OSE cells that were immortalized by sequential transfection with SV40 T antigen (called IOSE29 cells) and human telomerase reverse transcriptase (T29 cells), followed by full transformation with oncogenic H-RasV12 (T29H; ref. 10). By analogy to most cells in the EOC mass, neither IOSE-29 nor T29 cells expressed L1, whereas the latter was clearly detectable in isogenic, fully transformed T29H cells (Supplementary Fig. S3). In agreement with the observations in patient-derived specimens, these findings supported the notion that the expression of L1 is specifically associated to a highly malignant phenotype.

The Functional Role of L1 in Nontransformed versus Neoplastic Ovarian Epithelial Cells

Cell proliferation. To verify the hypothesis of a dual role of L1 in normal versus transformed OSE, we used gain-of-function and loss-of-function strategies in cells derived either from normal OSE or from ovarian carcinoma. Primary OSE cells are difficult to isolate and to maintain in culture, and they rapidly undergo cellular senescence (13). To overcome this problem, as a model for nontransformed ovarian epithelium, we used cells
isolated from normal OSE stably transfected with the SV40 early region, containing large T and small t antigens (SV40-TAg). This procedure has been reported to prolong the life span of OSE cells without inducing a tumorigenic phenotype (9, 10, 14, 15). In contrast with OSE cells in vivo, L1 was undetectable in four different lines of SV40-TAg–transfected OSE cells (Supplementary Figs. S3 and S4A), possibly due to the inactivation of L1-regulating transcription factors by SV40-TAg (see Discussion).

For the characterization of L1 activity in nontransformed OSE cells, we selected HIO-80 (called HIOSE/A; ref. 9) and IOSE80 (called HIOSE/B; ref. 10) cells to perform gain-of-function studies. Upon adenoviral transduction with the cDNA for human L1, HIOSE cells exhibited a remarkable expression of L1 which was correctly localized at the cell surface (Supplementary Fig. S4A). To study the role of L1 ovarian carcinoma cells, we selected two cell lines: IGROV1 cells, which express high levels of L1 (Supplementary Fig. S4B), and OVCAR3 cells, where L1 is almost undetectable (Supplementary Fig. S4C). The former cell line was transfected with siRNA oligonucleotides that have been successfully used to reduce L1 expression in human colon cancer cells (5). Indeed, the levels of L1 in transfected IGROV1 were dramatically decreased compared with control cells transfected with siRNA oligonucleotides targeting the mRNA for green fluorescent protein (GFP; Supplementary Fig. S4B). Instead, OVCAR3 cells were transduced with adenovirally expressed human L1 or with GFP (Supplementary Fig. S4C).

We first determined the effect of L1 on serum-induced proliferation of HIOSE and IGROV1 cells. After serum starvation, cells were stimulated with fetal bovine serum (FBS) and cell growth was determined every 24 h. The forced expression of L1 showed no significant effect on either HIOSE/A or HIOSE/B cells (Fig. 2A, top left), indicating that L1 is not involved in the proliferation of this cell type. In contrast, IGROV1 cells exhibited a marked reduction in cell proliferation upon abrogation of L1 expression compared with cells transfected with the siRNA for GFP (Fig. 2A, top right). The decrease in the proliferation rate of L1-deficient IGROV1 cells was further validated by a reduction in serum-induced DNA replication, as shown by BrdUrd incorporation (Fig. 2B, top right).

We further confirmed the role of L1 in serum-induced proliferation in ovarian cancer cell lines. After serum starvation, cells were stimulated with fetal bovine serum (FBS) and cell growth was determined every 24 h. The forced expression of L1 showed no significant effect on either HIOSE/A or HIOSE/B cells (Fig. 2A, top left), indicating that L1 is not involved in the proliferation of this cell type. In contrast, IGROV1 cells exhibited a marked reduction in cell proliferation upon abrogation of L1 expression compared with cells transfected with the siRNA for GFP (Fig. 2A, top right). The decrease in the proliferation rate of L1-deficient IGROV1 cells was further validated by a reduction in serum-induced DNA replication, as shown by BrdUrd incorporation (Fig. 2B, top right).
incorporation assays (Supplementary Fig. S5). To confirm that the inhibitory effect depended on L1, we incubated parental IGROV1 cells with CE7, a monoclonal antibody that has been reported to repress the tumorigenicity of ovarian cancer cells in immunodeficient mice (16). In agreement with the data on L1 knockdown cells, the CE7 monoclonal antibody, but not an isotype-matched irrelevant antibody, decreased the proliferation of IGROV1 cells (Fig. 2A, bottom left). As these results indicated that L1 is required for EOC cell proliferation, we asked whether it is also sufficient. To address this question, we determined the effect of forcing L1 expression in OVCAR3 cells on their growth capacity after serum stimulation. Indeed, the proliferation rate of OVCAR3-L1 cells was markedly higher than control, GFP-expressing cells (Fig. 2A, bottom right). Overall, these findings implicated L1 in the proliferation of neoplastic, but not normal, OSE cells.

To gain insight into the mechanism underlying L1-dependent ovarian cancer cell proliferation, we focused on the Erk1/2 pathway, known to mediate cell proliferation in several experimental models. As many transformed cell lines, serum-starved IGROV1 exhibited constitutive activation of Erk1/2 that, however, was markedly reduced upon loss of L1 (Fig. 2B, right). Both control and L1 knockdown IGROV1 cells responded to serum stimulation with an increase in Erk1/2 activation. In contrast, no constitutive activation of Erk1/2 was observed in HIOSE cells, and this was not changed by the forced expression of L1 (Fig. 2B, left). Erk1/2 activation in response to serum was retained in both control and L1-transfected HIOSE cells. These findings imply that L1 regulates the Erk1/2 signaling pathway in neoplastic, but not normal, ovarian epithelial cells.

**Crosstalk of L1 with Fibroblast Growth Factor Receptor Signaling**

Because L1 enhanced the growth of serum-stimulated ovarian carcinoma cells (Figs. 2A and 3A) but was unable to stimulate the proliferation of serum-starved cells (Fig. 3B and C), it is conceivable that L1 synergizes with a signaling machinery elicited by growth factors contained in the serum. In an attempt to identify such signaling partners, we focused on two growth factor receptors that have been previously implicated in L1 function in neurons, the fibroblast growth factor receptor (FGFR) and the epidermal growth factor receptor (EGFR; refs. 17, 18). Aberrant expression and/or
activities of both receptors have been associated with ovarian malignancy (19–21). Serum-induced proliferation of OVCAR-3 cells expressing either GFP or L1 was determined after a pretreatment of the cells with PD173074 or AG1478, which specifically inhibit FGFR and EGFR activity, respectively. Interestingly, PD173074 specifically abrogated the positive effect of L1 on serum-induced proliferation, without affecting the growth of OVCAR-3-GFP cells (Fig. 3A), thus pointing to a functional cooperation between L1 and FGFR. In contrast, the growth of both GFP-expressing and L1-expressing OVCAR-3 cells was reduced by AG1478 to a very low level (Supplementary Fig. S6A), implicating EGFR in the proliferative capacity of OVCAR-3 cells but ruling out any specific effect on L1-dependent proliferation. To confirm the interplay between L1 and FGFR, serum-starved OVCAR3-GFP or OVCAR3-L1 cells were stimulated with FGF-2 and subjected to cell proliferation assay. Notably, only L1-expressing cells exhibited a remarkable proliferative response to FGF-2 (Fig. 3B), thus confirming that L1 cooperates with FGFR signaling. In agreement with the data on AG1478, EGF stimulated the proliferation of both OVCAR3-GFP and OVCAR3-L1 cells to the same extent (Supplementary Fig. S6B), implying the lack of a crosstalk between L1 and EGFR. The role of L1 in FGFR activity was also confirmed in IGROV1 cells by a loss-of-function approach. Indeed, the abrogation of L1 expression in these cells led to a marked decrease in FGF-induced proliferation, compared with cells transfected with a control siRNA (Fig. 3C).

**Apoptosis Resistance**

Previous work implicated L1 in the protection of ovarian carcinoma cells from apoptosis (22). To verify whether this antiapoptotic response occurs also in nontransformed OSE cells, we compared the effect of the drug staurosporine, which induces caspase-mediated apoptosis, in HIOSE and IGROV1 cells. The activation of caspase-3 after staurosporine treatment, a widely used readout for apoptosis, was monitored by immunoblotting with an antibody specific for the activated (i.e., cleaved) form of caspase-3. Stauorosporine induced caspase-3 cleavage in L1-negative HIOSE cells with low efficiency, with maximal effect at 2 h of treatment in HIOSE/A and at 4 h in HIOSE/B cells. However, upon expression of L1, the proapoptotic effect of staurosporine increased remarkably in both cell lines (Supplementary Fig. S7). In contrast, L1 counteracted staurosporine-induced apoptosis in IGROV1 cells. Indeed, whereas control cells showed no caspase-3 cleavage during a 4-h treatment, cleaved caspase-3 in response to staurosporine became evident upon siRNA-mediated down-regulation of L1 (Supplementary Fig. S7). Thus, L1 acted as a survival factor in EOC cells, confirming and extending previous observations (22), while it enhanced drug-induced apoptosis in nontransformed OSE cells.

**Cell-Cell and Cell-Matrix Adhesion**

L1 has been originally characterized as an adhesion molecule that promotes the physical interaction between adjacent cells. In addition, cell-cell adhesion has long been known to affect tumor malignancy and, in particular, may counteract the peritoneal dissemination of ovarian carcinoma (23). Based on these considerations and on our observation that, in EOC tissues, high levels of L1 correlated with a less cohesive phenotype (e.g., see Supplementary Fig. S2B), we checked whether L1 regulates cell-cell adhesion in nontransformed versus neoplastic ovarian epithelial cells. As shown in Fig. 4A, the forced expression of L1 in either HIOSE/A or HIOSE/B...
cells enhanced their intercellular adhesion. We tested the effect of L1 on both calcium-independent and dependent cell-cell adhesion. Whereas the former is mediated by Ig-CAMs, calcium-dependent intercellular adhesion is initiated by members of the cadherin family (2). Thus, the fact that L1 stimulated also calcium-dependent adhesion in HIOSE cells (Fig. 4A) suggested a functional interaction with cadherins in this cell type. In contrast, L1 exhibited a negative effect on cell-cell adhesion in IGROV1 cells, in that L1 knockdown cells formed ~ 3.5-fold and 10-fold more clusters than control cells in calcium-independent and calcium-dependent conditions, respectively (Fig. 4B). Therefore, L1 stimulates cell-cell adhesion in HIOSE cells, whereas it exerts an inhibitory effect on intercellular adhesion in IGROV1 cells.

L1 has been reported to interact with integrins, the key regulators of cell adhesion to the extracellular matrix (24). Hence, we asked whether L1 affected cell-matrix adhesion in nontumorigenic and neoplastic ovarian epithelial cells. Adhesion assays on different extracellular matrix components revealed that L1 stimulates cell-matrix adhesion, although the efficiency of this proadhesive activity and the specific integrins involved (as reflected by the adhesion to specific ECM components) show some differences between HIOSE and IGROV1 cells (Supplementary Fig. S8).

**Cell Invasion**

Previous studies have shown that L1 is implicated in the migration of ovarian carcinoma cells (7). However, it has remained elusive whether L1 also modulates the invasion of tumor cells through a three-dimensional extracellular matrix, a key step in cancer progression. To address this issue, we subjected L1-positive and L1-negative HIOSE and IGROV1 cells to invasion assays through Matrigel, a reconstituted basement membrane. Both HIOSE/A and HIOSE/B cells exhibited a weak invasive activity that was not significantly affected by the ectopic expression of L1 (Fig. 5A, left and middle columns). In contrast, L1 was required for Matrigel invasion by IGROV1 cells, as shown by the ~ 75% reduction of invasive activity upon ablation of L1 expression (Fig. 5A, right columns). Given the biological implications of these findings for the progression of ovarian carcinoma, we used the L1-negative OVCAR-3 cellular model to verify whether L1 was also sufficient for ovarian cancer cell invasion. As shown in Fig. 5B, the forced expression of L1 resulted in a 4-fold increase in OVCAR-3 cell invasion, confirming that this adhesion molecule contributes to the malignant properties of ovarian cancer cells.

Based on our observation of a L1/FGFR crosstalk in ovarian cancer cell proliferation, we asked whether a similar mechanism was involved in L1-induced OVCAR-3 cell invasion. Indeed, a pretreatment with the FGFR inhibitor PD173074 efficiently inhibited Matrigel invasion of L1-expressing OVCAR-3 cells to the level of control, untreated cells, whereas the invasion of OVCAR-3 cells was not affected (Fig. 5B). This specifically implicates FGFR signaling in L1-induced invasion. In contrast, the EGFR inhibitor AG1478 reduced the invasive capacity of both OVCAR-3 and OVCAR3-L1 cells by ~ 3.5-fold (Supplementary Fig. S6C), which suggests that EGFR is involved in ovarian cancer cell invasion in a L1-independent manner.

In an attempt to identify the signaling mediator(s) of L1-dependent tumor cell invasion, we assessed the Matrigel invasion of L1-transfected OVCAR-3 cells in the presence of specific inhibitors of either the Erk1/2 or the phosphoinositide 3-kinase (PI3K) pathways, two major biochemical cascades previously implicated in cancer cell malignancy. Both U0126 and LY294002, which inhibit Erk1/2 and PI3K activity, respectively, efficiently repressed the invasion of L1-expressing OVCAR-3 cells (Fig. 5B). These findings indicate that L1 enhances the invasive potential of ovarian carcinoma cells via the Erk1/2 and PI3K signaling pathways.

**Transendothelial Migration**

Ovarian carcinoma disseminates predominantly through detachment of tumor masses from the primary site, which then spread into the peritoneal cavity and/or colonize peritoneal organs (25). However, a significant proportion of ovarian carcinomas forms metastases in the retroperitoneal lymph nodes, most likely disseminating through the lymphatic circulation (26, 27). To verify whether L1 is implicated in this route of EOC metastatic spread, we assayed for the ability of L1 to modulate the transmigration of wild-type versus L1-deficient IGROV1 cells through a monolayer of lymphatic endothelial cells. We used HDLEC1 cells, a lymphatic endothelial cell line, whose life span was prolonged by telomerase expression, and HMEC-1 cells, a widely used endothelial cell line that was recently reported to express several lymphatic endothelial markers (28). Reducing L1 expression in IGROV1 cells caused a 2-fold decrease in their ability to cross the lymphatic endothelial barrier constituted by either HMEC-1 or HDLEC1 cells (Fig. 5C). An
even stronger effect was observed on the transmigration through blood vessel endothelial cells (Fig. 5C). Therefore, L1 is required for the transendothelial migration of ovarian carcinoma cells, emerging as a potential player in the formation of EOC lymph node metastasis.

**Discussion**

This study reports for the first time that the adhesion molecule L1 exerts different functions in nontransformed versus neoplastic OSE cells, promoting a nonmalignant phenotype in the former and an invasive one in the latter. Previous studies have implicated L1 in EOC progression, based on the correlation between L1 expression and poor prognosis in patients (6), an observation that we have confirmed and extended in the present report, and on the L1-dependent growth of EOC xenografts in the peritoneum of immunodeficient mice (16). However, the role of L1 in OSE cells has not been addressed, likely due to previous negative results on L1 expression in this tissue. Indeed, the groups of Altevogt and Fogel have reported the absence of L1 in normal OSE (6, 29), a result that is in sharp contrast with our data. Those studies were performed using two monoclonal antibodies (L1-11A and L1-14-10, both raised against the ectodomain of L1) different from the antibodies used in our screening, which probably accounts for the discrepancy between the results of Altevogt and Fogel and ours. This highlights the importance of the antibody selection when analyzing the expression and localization of L1, also based on the notion that L1 can undergo extensive posttranslational modifications (including glycosylation, proteolytic cleavage, and ectodomain shedding; ref. 30), which probably change its immunoreactivity. Overall, the finding that OSE cells do express L1 is noteworthy because, combined with the specific L1 enrichment in highly invasive EOC cells, it was at the origin of our hypothesis that L1 plays a dual role in nontransformed versus neoplastic OSE cells.

In nontumorigenic OSE cells, L1 supported cell-cell adhesion and enhanced drug-induced apoptosis, whereas it showed no effect on cellular processes associated with tumor malignancy. In contrast, ovarian cancer cells exhibited L1-dependent cell proliferation, invasion, resistance to apoptosis, and transendothelial migration, all representing important steps in cancer progression, whereas cell-cell adhesion was repressed by L1. The inhibitory effect of L1 on intercellular adhesion is not restricted to ovarian cancer cells, as it was recently reported in the breast cancer cell line MCF7, where L1-mediated disruption of adherens junctions resulted in enhanced cell motility (31). On one hand, our findings confirm and extend previous observations on the role of L1 in enhancing the malignant phenotype of ovarian carcinoma. For example, L1 has been reported to support the i.p. growth of ovarian cancer cells in immunodeficient mice (16) and to induce their migration and resistance to apoptosis (7, 22). On the other hand, our results highlight a novel function of L1 in nontumorigenic OSE cells, namely the induction of both calcium-dependent and independent cell-cell adhesion. Based on the notion that intercellular adhesion, and in particular the calcium-dependent one, efficiently represses tumor invasion (2), it is tempting to speculate that L1 contributes to inhibit or restrict the malignant transformation of OSE cells. In agreement with this hypothesis, L1 is down-regulated in the majority of the tumor cells during EOC development (this study and ref. 6). Yet, high levels of L1 are present in less cohesive cells within the tumor mass, as well as at the tumor-stroma interface, namely where cancer cells are actively invading the surrounding tissue. Overall, the picture emerging from the expression pattern of L1 in EOC and from our functional studies in cultured cells is consistent with a model, whereby L1 enhances intercellular adhesion in OSE while it acts as a tumor promoter in advanced EOC.
The cellular and molecular factors that determine the changes in L1 expression during EOC progression remain elusive. In advanced colon carcinoma, the Wnt/β-catenin/TCF pathway has been proposed to induce the expression of L1 at the invasive front of the tumor (5). Unlike colon carcinoma, whose normal counterpart is negative for L1 (5), the latter is found both in normal OSE and in a subset of EOC cells, implying that different mechanisms could regulate the expression of L1 in different tumors. Because most of the tumor cells in EOC masses show no L1 expression, ovarian tumorigenesis is accompanied by a general loss of L1. Such a loss could result from the inactivation of tumor-suppressing genes, such as p53, an event occurring in 50% to 70% of advanced ovarian cancers (32). Indeed, an inactivating mutation in the p53 gene is accompanied by the down-regulation of L1 in small cell carcinoma of the prostate.8 Along the same line, we report the loss of L1 in OSE cells expressing the SV40 T antigen, a well-characterized antagonist of p53 function (33). The presence of L1 at the invasive front of more advanced EOC could then depend on its reexpression as part of the transition toward an invasive phenotype. Based on our results in Ras-transformed OSE cells (see Supplementary Fig. S3) and on the frequency of oncogenic Ras mutations in advanced EOC (34), it is conceivable that this oncogene is causally involved in the expression of L1 in invasive EOC cells. Along this line, we have observed induction of L1 in OVCAR3 cells upon forced expression of activated Ras.9 At the cellular level, one possibility is that microenvironment-derived factors induce L1 expression in the cancer cells located at the tumor-stroma interface. Alternatively, the L1-positive cells at the edge of EOC might derive from the selection of a subset of transformed OSE cells that have maintained the expression of L1, accompanied by a functional switch of the protein to a proinvasive activity, thus enhancing tumor malignancy.

The dual role of L1 in OSE cells indicates that the cellular context and, in particular, the acquisition of a transformed phenotype, has a major effect on the function of L1 and can actually switch it from a bona fide cell-cell adhesion molecule to a tumor-promoting factor. The molecular events that determine this functional switch remain unknown. An attractive candidate as a mediator of L1’s effect on EOC development is the FGFR, given that (a) an aberrant FGFR signaling has been associated to EOC malignancy (19, 20) and (b) the crosstalk between L1 and FGFR has long been described in neuronal cells, where it stimulates axonal growth (17), although the physical association between the two molecules has not been reported. Our results showed for the first time a functional interaction of L1 with FGFR in ovarian cancer cells, where it plays an important role in L1-dependent cell proliferation and invasion. Thus, interfering with the L1/FGFR crosstalk might prove a suitable therapeutic approach for the treatment of EOC. In agreement with our observations, the adhesion molecule N-cadherin has been shown to potentiate FGFR signaling in breast cancer cells by favoring a sustained activation of the receptor by FGF-2 (35). It is noteworthy that the crosstalk of an adhesion molecule with FGFR can also lead to the repression of FGF-induced signaling, as we have recently reported for another Ig-CAM, neural cell adhesion molecule, that abolishes the cellular response to FGF-2 in fibroblasts (36). Hence, the outcome of the adhesion molecule/FGFR interaction is likely to depend on the specific adhesion molecule involved and on the cellular context. On the other hand, also the repertoire of growth factor receptors involved in crosstalk with L1 seems to be cell type–specific, because, unlikely previous observations in neurons (18), we obtained no evidence of L1 interacting with the EGFR signaling machinery in ovarian cancer cells.

Other signaling mediators that have been implicated in L1-induced axonal growth (37) and, based on our studies, are also involved in L1-dependent ovarian cancer progression include mitogen-activated protein kinase and PI3K pathways. But why are these signaling cascades activated by L1 specifically in ovarian cancer and not in OSE cells? A possible scenario is that the repertoire of proteins interacting with L1 changes upon neoplastic transformation of OSE cells. Indeed, many studies on neuronal systems have documented the heterophilic interactions of L1 with a broad spectrum of molecules. The interacting partners of L1 include several components of the extracellular matrix, cell surface molecules, such as EGFR, neuropilin, and various integrins (besides FGFR, as mentioned above), intracellular signaling effectors, such as Src, Numb, and RanBPM, and cytoskeletal components, such as ankyrins and ezrin (reviewed in refs. 3, 38). Many of these molecules are involved in cellular processes that, once deregulated, contribute to cancer progression, thus providing a potential basis for L1-dependent function in EOC. For example, FGFRs are abundantly expressed in EOC (19, 20, 39), and our data support the hypothesis that the simultaneous expression of L1 leads to excessive FGFR signaling that, in turn, enhance tumor invasion.

In summary, we have shown that L1 plays a dual role in OSE cells, consistent with a tumor-suppressive function in nontransformed cells and with a proinvasive function in cancer cells. The characterization of the cellular and molecular determinants responsible for this shift in L1 activity will contribute to the identification of novel regulatory mechanisms implicated in EOC progression, hopefully providing new therapeutic targets for this neoplastic disease.

Acknowledgments

Received 7/30/2007; revised 11/8/2007; accepted 12/19/2007.

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We thank V. Lemmon, M. Schachner, K. Blaser, T.J. Lawley, A. Insinga, R. Nisato, and M. Pepper for providing antibodies and cell lines.

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