The L1 Cell Adhesion Molecule Is Induced in Renal Cancer Cells and Correlates with Metastasis in Clear Cell Carcinomas

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ABSTRACT

Purpose: The L1 cell adhesion molecule is overexpressed in many human carcinomas. The objectives of the study were to provide a comprehensive description of L1 distribution in human kidney and to establish the prognostic relevance of L1 expression in renal cell carcinomas (RCC).

Experimental Design: Using two antibodies to the extracellular part and the cytoplasmic domain, respectively, we first compared L1 expression in normal kidney and renal tumors of diverse histopathologic origin, then we studied L1 expression together with tumor stage, grade, molecular prognostic markers, and metastatic behavior.

Results: In normal kidney, L1 immunoreactive with both antibodies was expressed in all epithelial cells originating from the ureteric bud except for intercalated cells. In renal tumors, L1 was mainly detected in those originating from cells that do not express L1 in the normal kidney [i.e., 33 of 72 clear cell RCC (ccRCC) and 25 of 88 papillary RCC (papRCC)]. Both in ccRCC and papRCC, L1 reacted only with the antibody to the extracellular domain, suggesting that the protein was truncated. In these carcinomas, L1 expression was strongly correlated with Ki-67 proliferation index (ccRCC, \( P = 0.0059 \); papRCC, \( P = 0.0039 \)), but only in ccRCC, the presence of L1 was associated with the risk of metastasis (\( P = 0.0121 \)). This risk was higher if cyclin D1 was concurrently absent in tumor cells (\( P < 0.0001 \)). The L1\(^+\) cyclin D1\(^-\) profile was an independent prognostic factor of metastasis occurrence in multivariate analysis (\( P = 0.0023 \)).

Conclusion: We have found a combination of markers that can serve to identify a subgroup of high-risk patients with ccRCC that may require more aggressive therapies.

INTRODUCTION

Renal epithelial tumors have different histologic origin, metastatic potential, and clinical outcomes (1). Renal cell carcinoma (RCC) represents 80% of renal tumors in adults and is characterized by lack of early warning signs, by frequent metastasis, and by resistance to currently used treatments (1, 2). Therefore, identification of molecular markers of tumor aggressiveness is urgently needed. In a variety of human malignancies, tumor progression is associated with changes in cell adhesion molecule (CAM) expression (3). For example, previous studies in clear cell RCC (ccRCC) showed that the expression of the standard CD44 (CD44s), \( \alpha_5\beta_1 \) or \( \alpha_v\beta_3 \) integrins, or the down-regulation of cadherin-6 or E-cadherin were associated with a poor prognosis (4–7). On the other hand, transcripts of the vascular cell adhesion molecule-1 (VCAM-1) gene were the most predictive of survival in a recent gene expression profiling study of metastatic kidney cancers (8). These findings were not unexpected as CAMs do not only keep cells in close contact, but most interactions of CAMs with their ligands induce the transmission of signals regulating cell differentiation, proliferation, or migration (9).

The L1 cell adhesion molecule (CD171) is a multidomain membrane glycoprotein of the immunoglobulin superfamily expressed in neural, hematopoietic cells, and certain epithelial cells (10–13). L1 is also overexpressed by a variety of highly malignant tumors (14–18). L1 promotes many cellular activities by interacting through its extracellular domain with other CAMs, extracellular matrix molecules, and signal receptors (19, 20). In addition to being expressed on the cell surface, the L1 ectodomain can be cleaved by a metalloproteinase ADAM10 and by plasmin (21–23). Soluble L1 is able to stimulate cell migration or survival through autocrine/paracrine binding to integrins (24, 25). Soluble L1 seems to be important for tumor progression in glioma (26), melanoma (27), uterine, and ovarian carcinoma (14). In the kidney, L1 plays a key role in branching morphogenesis of the ureteric bud and in later maturation of the renal papilla (27, 28). Its renal localization has been well established in rabbit but not in human (27).

The aims of the present study were 3-fold: first, to provide a comprehensive description of L1 distribution in human kidney; second, to analyze the prevalence of L1 expression in a large number of archival renal tumors; third, to establish the prognostic relevance of L1 expression by reviewing the available clinical records.

MATERIALS AND METHODS

Tissue Specimens. All specimens were collected during the period 1989 to 2002 and retrieved from the departments of
Pathology, in Tenon, Cochin, Lariboisiere, and Foch hospitals (Paris, France). The unaffected pole of 10 kidneys removed for a RCC was used as a source of normal renal tissue. Six embryonic and fetal kidneys were from therapeutic abortion material. A total of 308 paraffin-embedded renal tumors, including ccRCC (n = 72), papillary RCC (papRCC, n = 88), chromophobe RCC (CHRO, n = 48), carcinoma of the collecting ducts of Bellini (COLL, n = 6), and renal oncocytoma (ONCO, n = 94), were obtained from patients who underwent radical nephrectomy. Follow-up was available for 103 cases (52 ccRCC and 51 papRCC) from 2 to 133 months (mean, 34.7 months) after nephrectomy. All the tumors were reviewed by two pathologists (Y.A. and C.B.) and the histologic types were determined according to the WHO classification (29). Adult renal cancers were staged according to tumor-node-metastasis classification and examined for nuclear grade (1-4 according to Fuhrman classification; refs. 30, 31). The clinicopathologic features of the renal tumors are shown in Table 1. All tissue and clinical data were obtained after informed consent of the patients.

In Situ Hybridization. Paraffin sections of normal adult renal tissue were cut at 4 μm thickness, mounted on superfrost plus slides, and subjected to in situ hybridization. We used digoxigenin-labeled (Boehringer-Mannheim, Mannheim, Germany) hybridizing (antisense) and nonhybridizing (sense) RNA probes transcribed from L1 cDNA subcloned in PSTblue-1 vector plasmid (Novagen, Madison, WI). Sections were then treated with anti-digoxigenin-alkaline phosphatase, and developed with 4-nitroblue tetrazolium chloride (Sigma-Aldrich). Alternatively, in situ hybridization was done using L1-specific FITC-labeled Hybriprobe kit according to the manufacturer’s instructions. Except for Ki67, a minimum of 10% positive nuclei were counted to evaluate the percentage of positive nuclei, defined as the proliferative index. Two embryonic kidneys were also investigated in frozen sections and processed manually for L1 immunostaining as described in prior publication (27). Semithin cytosections of adult renal tissue were processed as described previously (27).

Statistical Analysis. The statistical analysis was done with StatView Software for Windows (version 5.0; SAS institute, Inc., Cary, NC). Correlation among L1 expression, histologic types, tumor stage, nuclear grade, and immunohistochemical markers were evaluated using Χ² test and Student’s t test. The Kaplan-Meier method was used to analyze the disease-free survival and assess the risk of metastasis according to the following variables: stage, nuclear grade, L1, Ki-67, EGFR, and cyclin D1 expression. Statistical differences between the groups were determined with the log-rank test. A multivariate analysis using the Cox proportional hazards regression model was used to test for independent prognostic value. Because an important finding was the induction of L1 in ccRCCs and papRCCs, L1 was considered as a dichotomous variable (i.e., present or absent) in all statistical analyses.

Table 1: Clinicopathologic features of the renal tumors

<table>
<thead>
<tr>
<th>N</th>
<th>ccRCC</th>
<th>papRCC</th>
<th>chroRCC</th>
<th>oncoRCC</th>
<th>collRCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (range) (years)</td>
<td>63 (24-92)</td>
<td>59 (18-82)</td>
<td>64 (29-88)</td>
<td>64 (24-87)</td>
<td></td>
</tr>
<tr>
<td>Gender M/F</td>
<td>51/21</td>
<td>75/13</td>
<td>25/23</td>
<td>27/24</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>pT1</td>
<td>pT2</td>
<td>pT3</td>
<td>pT4</td>
<td></td>
</tr>
<tr>
<td>Nuclear grade</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cases with follow-up</td>
<td>52</td>
<td>51</td>
<td>na</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Follow-up (months) mean</td>
<td>26</td>
<td>45</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td>13/52</td>
<td>11/51</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: M/F, male/female; RCC, renal cell carcinoma; cc, clear cell; pap, papillary; chro, chromophobe cell; onco, oncocytoma; coll, collecting duct; na, not available. *Age and gender for patients with oncocytoma were available only for 51 of them.

Antibodies. The primary monoclonal antibodies (mAb) used in this study were mAb272, an IgG1 (1:200 dilution of ascitic fluid) directed against the L1 extracellular domain (the generation of the mouse mAb272 and its specificity for L1 have been described previously; ref. 27); anti-Ki67 (clone MIB-1, 1:150 dilution, DAKO, Glostrup, Denmark); anti-epidermal growth factor receptor (EGFR, ready-to-use PharmDx-EGFR kit, DAKO); anti-cyclin D1 (clone DCS-6, 1:100 dilution, DAKO). The polyclonal antibodies were goat anti-L1 directed against the cytoplasmic domain (clone sc-1508, 1:100 dilution, Chemicon International) and rabbit anti-ADAM10 (1:50 dilution, Chemicon International).

Immunohistochemistry. Sections of normal embryonic and adult renal tissues and collecting duct carcinomas were prepared from original paraffin-embedded tumor blocks. In addition, four tissue microarrays, bringing together oncocytoma, conventional (clear cell), papillary, and chromophobe RCC cases, respectively, were constructed for the immunohistochemical study. H&E-stained sections from original block were used to select a representative tumor area for each case. Triplicate neighboring tissue cores with a diameter of 0.6 mm were punched from selected area of the donor block and arrayed on a new recipient paraffin block, with a precision instrument (Tissue-arrayer, Beecher Instrument, Silver Springs, MD). For all paraffin-embedded tissues, 4-μm sections were cut and placed on superfrost plus slides. Slides were microwaved thrice 5 minutes at 300 W for antigen retrieval. The sections were then processed on an automated instrument (Ventana Nexes; Ventana Medical Systems, Paris, France) for immunostaining using an indirect biotin avidin system, the Ventana Basic 3,3′-diaminobenzidine detection kit (Ventana Medical Systems) according to the manufacturer’s instructions. Except for Ki67, a minimum of 10% positive cells was necessary to assess as positive the immunostainings.

Tumor staining intensity score was then evaluated by the pathologist (Y.A.) using a semiquantitative scale: 0, null; +, mild; ++, moderate; ++++, intense. For Ki67, 1,000 nuclei were counted to evaluate the percentage of positive nuclei, defined as the proliferative index. Two embryonic kidneys were also investigated in frozen sections and processed manually for L1 immunostaining as described in prior publication (27). Semi-thin cryosections of adult renal tissue were processed as described previously (27).
RESULTS

L1 in Mature and Developing Normal Human Kidney. The adult kidney is composed of single units, the nephrons, that produce urine drained by the collecting duct system. Adult renal epithelia derive from two sources. Collecting duct system epithelia develop by arborization of the initially unbranched ureteric bud, whereas nephron epithelia result from mesenchyme-to-epithelial transition of the induced metanephric blastema (32). Immunohistochemistry experiments done in developing kidneys at different ages (8, 10, 17, 18, 25, and 27 weeks of gestation) revealed that L1 was first expressed in the ureteric bud and then in its derivatives, the developing collecting ducts and the most distal part of the newly formed primitive nephrons, the future connecting segments (Fig. 1A). No staining was present in mesenchymally derived epithelium (glomeruli, proximal, and distal tubules).

In situ hybridization and immunohistochemistry were used to analyze L1 mRNA and protein expression in mature renal tissue. The combination of the two methods was necessary to ascribe membrane expression to local synthesis. In the human adult kidney cortex, in situ hybridization experiments revealed L1 mRNA signals in collecting ducts (CD) and connecting tubules (Fig. 1B). No expression was seen in glomerular structures, proximal tubules, loops of Henle, distal tubules, and the vascular smooth muscle or endothelial cells of the vessels. The same pattern of expression was observed using immunohistochemistry methods (data not shown). In the adult kidney, the terminally differentiated CD epithelium is composed of principal cells and of intercalated cells (IC; ref. 33). Detailed immunohistochemistry experiments done on semithin cryosections showed that L1 was not expressed on IC cells but strong reactivity was seen on the basolateral cell membrane of the principal cells (Fig. 1C). Serial adjacent sections confirmed the absence of L1 staining in CD cells that expressed H+/ATPase, a marker for IC cells (data not shown). At the cellular level, expression of L1 mRNA closely mirrored that of the protein detected by immunohistochemistry, with lack of positive signal in IC cells (Fig. 1D). In the deep segments of the inner medullary CD where IC and ciliated principal cells are replaced by progressively taller cells, L1 protein was expressed only on the lateral membrane of these tall cells (Fig. 1E) and L1 mRNA was present in each of these cells in the inner medullary CD (Fig. 1F). To investigate L1 expression in mature and developing normal human kidney, we used two antibodies: one to the extracellular domain (mAb272) and the other to the cytoplasmic domain (Chemicon International, Santa Cruz, CA). Because the same pattern of expression was observed with both antibodies, only data obtained with mAb 272 are presented in Fig. 1.

L1 Expression in Human Renal Tumors. To evaluate the prevalence of L1 protein expression in renal tumors, we examined 308 tumors mostly in tissue microarrays using mAb 272. The frequency of L1 expression in renal tumors is summarized in Fig. 2, and its cellular localization is shown in Table 2. L1 expression was induced in ccRCCs (47%), and to a lesser extent in papRCCs (28%) which are known to originate from proximal and distal tubules, respectively, and comprise the most frequently invasive renal neoplasms (1). By contrast, L1 was rarely induced in chromophobe cell carcinomas (2%) and oncocytomas (8%), which form a group of tumors originating from L1-negative IC cells and are known to be low malignant or benign (1). In ccRCCs and oncocytomas, L1 was seen at tumor cell membrane and in cytoplasm. In papRCCs, L1 was observed predominantly in cytoplasm and occasionally at cell membrane. In addition, L1 was observed in endothelial cells of the stroma, in three oncocytomas and two chromophobe cell carcinomas (in the absence of L1 in tumor cells), in eight ccRCCs (including three cases with concomitantly L1 positive tumor cells), and in none of the papRCCs. No significant L1-positive inflammatory cells were noticed within the stroma. On the other hand, L1 was...
weakly expressed in tumor cell cytoplasm of 5 of 6 (83%) collecting duct carcinomas that originate from medullary principal cells, which in their normal state, strongly express L1 at the basolateral or lateral membrane.

We also investigated the expression of L1 protein in ccRCCs, papRCCs, and oncocytomas using a polyclonal antibody to the cytoplasmic domain. In all L1-positive ccRCC and papRCC, L1 immunoreactivity was only observed with mAb 272 against the extracellular domain, whereas the polyclonal antibody did not stain, which suggests that L1 has lost at least a part of its cytoplasmic tail in these tumors (Fig. 3B-E). In contrast in oncocytoma (Fig. 3F and G) and in the normal part of the kidney, immunoreactivity was observed with both antibodies (Fig. 3H and I).

Association of L1 Expression with Established Tumor Markers. Because L1 was induced in ccRCCs and papRCCs, we focused on these two histologic subtypes and searched for correlation between L1 expression and prognostic markers. In ccRCCs, L1 expression was not correlated with the tumor stage or the nuclear grade. However, a correlation was observed among L1 expression, proliferation index Ki-67, and EGFR expression (Table 3). The Ki67 index was significantly higher in L1-positive tumors than in L1-negative tumors. This difference was still observed for low tumor stages (pT1/pT2) and low nuclear grades (1and/or 2). ADAM10 was rarely detected in ccRCCs and showed no correlation with L1 expression. We did not find any significant correlation between L1 and cyclin D1 expression. Previous studies had shown that cyclin D1 expression in ccRCCs was not correlated with proliferation index in ccRCC (34, 35). We addressed this issue in tumors classified according to L1 expression. In L1-negative ccRCCs, the proliferation index was significantly higher in cyclin D1–positive tumors (4 ± 0.5%) than in cyclin D1–negative tumors (2.2 ± 0.6%; P = 0.04). By contrast, in L1-positive ccRCCs, the proliferation index was lower in cyclin D1–positive tumors (7.1 ± 1.2%) than in cyclin D1–negative tumors (12.1 ± 4.9%), but the difference did not reach statistical significance because of large variance.

Fig. 2 L1 expression in renal tumors of diverse histologic types. L1 is more frequently expressed in clear cell (CC) and papillary carcinoma (PAP) than in chromophobe cell carcinoma (CHRO) and oncocytoma (ONCO; χ² test).

Table 2 L1 localization in renal tumors

<table>
<thead>
<tr>
<th>ccRCC</th>
<th>papRCC</th>
<th>chroRCC</th>
<th>onco</th>
<th>collRCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>22</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>M/C</td>
<td>9</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>15</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>E*</td>
<td>8 (3)*</td>
<td>0</td>
<td>2 (0)</td>
<td>3 (0)</td>
</tr>
</tbody>
</table>

Abbreviations: M, tumor cell membrane; M/C, tumor cell membrane and cytoplasm; C, tumor cell cytoplasm; E, endothelial cell; cc, clear cell; pap, papillary; chro, chromophobe cell; onco, oncocytoma; coll, collecting duct.

*Cases with L1 both in tumor and endothelial cells.

Fig. 3 Representative pictures of L1 immunohistochemistry staining in RCC. A, model of the domain structure of L1. mAb 272 is directed against the immunoglobulin domain of L1 molecule, whereas the polyclonal antibody used in this study is directed against the cytoplasmic domain. B-I, stained with hematoxylin and immunostained for L1 (brown). Clear cell carcinoma (B and C), papillary carcinoma (D and E), oncocytoma (F and G), and representative example of normal tissue from a kidney with ccRCC (H and I). Left, staining with mAb 272; right, staining with pAb. Original magnification × 200.
In papRCCs, L1 expression was not correlated with the tumor stage or the nuclear grade. Like ccRCCs, the papRCCs expressing L1 showed a higher Ki67 index than the ones without L1 (Table 4). There was no correlation between L1 and EGFR expression. Cyclin D1 and ADAM10 expression were significantly associated with L1 expression. No correlation was found between cyclin D1 and Ki67 index.

**Prognosis Factors for Metastasis.** Clinical follow-up data were available in 56 of 72 patients with ccRCCs (21 L1-negative tumors and 35 L1-positive tumors) and in 48 of 84 of those with papRCCs (34 L1-negative tumors and 14 L1-positive tumors) included in the tissue microarrays. In ccRCCs, univariate analysis showed that L1 within tumor and/or endothelial cells was associated with a higher risk of metastasis in comparison with the absence of detectable L1 (P = 0.01; Fig. 4A). When we considered separately the groups with L1 only in tumor or only in endothelial cells, the same association with metastasis was observed for both groups but without reaching statistical significance (P = 0.077) due to the number of cases. This analysis also indicated that the absence of detectable cyclin D1 (P = 0.01; Fig. 4B), as well as the nuclear grade and tumor stage (data not shown) were significantly associated with the risk of metastasis.

Because the previous analysis showed that ccRCCs with L1+/cyclin D1− profile have a high proliferation rate, we focused on the prognostic impact of this profile. The ccRCCs with L1 in tumor and/or endothelial cells and no detectable cyclin D1 were associated with a higher risk of metastasis in comparison with all other cases including profiles L1+/cyclin D1+, L1−/cyclin D1+, and L1−/cyclin D1− (P < 0.0001; Fig. 4C). We observed the same strong association with metastasis when we considered the cases with no cyclin D1 and L1 detected in tumor cells only. In multivariate analysis, L1 expression alone or cyclin D1 alone were not independent risk factors of metastasis. However, using a proportional Cox model, including tumor stage, nuclear grade, and L1+/cyclin D1+ profile as covariables, the L1+/cyclin D1− profile was found to be an independent prognostic factor for metastasis occurrence (P = 0.0023; Table 5).

By contrast to ccRCC, in papRCCs, L1 (Fig. 4D), cyclin D1, as well as ADAM10 were not associated with metastasis (data not shown). For the latter carcinomas, only tumor stage and nuclear grade were associated with the risk of metastasis (data not shown).

**DISCUSSION**

In this work, we have analyzed L1 expression in normal adult and fetal human kidney and a broad range of renal epithelial tumors. Although Meli et al. detected L1 in 5 of 15 renal carcinomas, the limited number of cases and the lack of clinical and morphologic data precluded any definitive conclusion (36). Furthermore, the lack of information on L1 expression in normal human kidney and during renal embryogenesis was an obstacle to correct interpretation of L1 expression in various types of renal tumors. Here we show that L1 is constitutively expressed in epithelial structures that originate from the mesonephric duct (i.e., the collecting duct system). L1 appear early during development within the ureter bud and its derivatives and persists in all cell types with the exception of intercalated cells in the terminally differentiated collecting duct. In the metanephric mesenchyme derivatives, L1 is expressed only in the connecting segment in the fetal and adult kidney. This tightly regulated expression of L1 is no longer observed in neoplastic conditions. L1 was weakly expressed in the cytoplasm of aggressive carcinomas of the collecting duct which strongly express L1 at the cell membrane in the normal kidney. On the other hand, we observed induction of L1 in tumors derived from cells that fail to express L1 in the normal tissue whatever adult or in earlier stages of fetal development. Indeed L1 was frequently detected in tumors originating from cells in the proximal and distal tubules, including ccRCC (47%) and papRCC (28%), and to a much lesser extent in oncocytomas (8%) and chromophobe.

### Table 4 Pathologic and immunohistochemical profile of papRCCs

<table>
<thead>
<tr>
<th></th>
<th>L1+ papRCC</th>
<th>L1− papRCC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>25</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Stages*</td>
<td>pT1/pT2 80%</td>
<td>84%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>pT3/pT4 20%</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>Nuclear grade*</td>
<td>1/2 76%</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3/4 24%</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>Ki-67*</td>
<td>(mean ± SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.8 ± 0.8%</td>
<td>1.1 ± 0.2%</td>
<td>0.0039</td>
</tr>
<tr>
<td></td>
<td>2.0 ± 0.6%</td>
<td>1.1 ± 0.2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.8 ± 2.8%</td>
<td>1.0 ± 0.5%</td>
<td>0.0426</td>
</tr>
<tr>
<td>Grade 1/2</td>
<td>2.6 ± 0.8%</td>
<td>1.0 ± 0.2%</td>
<td>0.0190</td>
</tr>
<tr>
<td></td>
<td>3.2 ± 1.8%</td>
<td>1.2 ± 0.4%</td>
<td></td>
</tr>
<tr>
<td>EGFR positive*</td>
<td>72%</td>
<td>57%</td>
<td>NS</td>
</tr>
<tr>
<td>ADAM 10 positive*</td>
<td>33%</td>
<td>4%</td>
<td>0.0002</td>
</tr>
<tr>
<td>Cyclin D1 positive*</td>
<td>56%</td>
<td>17%</td>
<td>0.0040</td>
</tr>
</tbody>
</table>

*Comparisons between L1-positive tumors and L1-negative tumors used the χ² test (dichotomous variables).  

*Comparisons between L1-positive tumors and L1-negative tumors used the Student’s t test (continuous variables).
cell carcinomas (2%), two groups of tumors derived from intercalated cells. In normal epithelial cells, L1 mainly showed plasma membrane localization. In some of our tested tumors (Table 2), L1 was aberrantly localized in the cytoplasm. In addition, we have detected a truncated form of L1 lacking a part of its cytoplasmic tail in ccRCC and papRCC exclusively. The biological basis and significance of these two anomalies which only occur in tumor cells is yet unclear and warrant further studies.

Because of the ectoexpression of L1 in a significant proportion of ccRCCs and papRCCs, we assessed whether the presence of L1 could predict progression and metastasis occurrence in these subtypes. L1 expression was not associated with traditional prognostic factors such as nuclear stage or tumor grade. However, univariate analysis revealed that the expression of L1 within tumor and or endothelial cells was associated with a higher risk of metastasis in ccRCC. Thus, L1 expression in ccRCC might be important for tumor biology. Indeed, ectoexpression of L1 may disturb normal intercellular communications and lead to the establishment of new adhesive interactions promoting cell survival and migration. Several studies have recently highlighted an important role of L1 in tumorigenesis of ovarian and uterine carcinoma, glioma, and melanoma (14, 15, 26) involving proteolytic cleavage of L1 ectodomain by ADAM10 or plasmin. The shed L1 can then trigger migration by binding to tumor cells via integrins, or alternatively soluble L1 can be immobilized and serve as a substrate for adhesion and migration of tumor cells (37). Our data do not support proteolytic cleavage of L1 by ADAM10 (Table 3) but other mechanisms may be involved as well in L1 processing. For example, increased basal shedding of L1 was recently detected in cells expressing the truncated form of L1 lacking the intracellular domain (38). This observation suggests that truncated form of L1 expressed in ccRCC might be released and deposited in the extracellular matrix.

Because L1 is a multidomain protein that may interact with a variety of other molecules, its function depends on coexpressed ligands. We found a strong correlation between L1 expression and EGFR in ccRCC. Constitutive activation of growth factor receptors was detected at high frequency in diverse human cancers (39), and in several studies, EGFR expression was associated with rapid tumor proliferation and poor prognosis (40, 41). L1 could activate growth factor receptors by direct interaction of the L1 extracellular domain with the receptor as shown for fibroblast growth factor receptor (42) and EGFR (43).

Table 5 Prognostic factors of metastasis occurrence in ccRCC in a multivariate analysis using a proportional Cox model

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Univariate (P) | Risk ratio (95% confidence interval) | P |
--- | --- | ---
Stage pT3/PT4 versus pT1/pT2 | 0.0041 | 2.754 (0.822-9.221) | 0.1004 |
Nuclear grade 3/4 versus grade 1/2 | 0.0005 | 3.876 (1.035-14.493) | 0.0443 |
L1+/cyclin D1− versus all others | <0.0001 | 7.353 (2.033-26.316) | 0.0023 |

Fig. 4 Kaplan-Meier analysis for metastasis occurrence. The disease free survival curves are plotted according to the metastasis occurrence, in ccRCC (A-C), in papRCC (D). Significance was assessed with the log rank test. C. In ccRCC, the stratification analysis shows that the L1+/cyclin D1− subset is associated with a significant risk of metastasis whereas all other profiles, including L1−/cyclin D1+, L1+/cyclin D1−, and L1−/cyclin D1+ are not. D. In papRCC, the risk of metastasis is not associated with L1 expression.

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One important step in the process of metastatic spread is the binding of tumoral cells to endothelial cells of the vasculature followed by extravasation. Given that tumoral cells expressing L1 can interact with multiple vascular or platelet integrins (24, 25), L1 induction could play an important role in transendothelial migration. In addition, L1 was observed also in endothelial cells of the stroma in some carcinomas. The endothelial L1 could interact with tumor cell integrins or other ligands, which might favor extravasation process.

Tumor progression is a multistep process in which tumor cells accumulate different abnormalities. The development of RCC from normal epithelia is also associated with altered expression of several genes whose products control cell division. In our study, the proliferation index Ki-67 was strongly higher in L1-positive tumor cells than in L1-negative tumor cells regardless of the histologic subtype. Proliferation index Ki-67 was shown in prior studies to have a prognosis impact in renal cancers (4), and the risk of metastasis in L1-positive tumors could partially be related to this higher proliferation activity. We assessed further the relationship between L1 and the cell cycle–related protein, cyclin D1 that regulates the G1-S phase transition (44). Thus far, a few studies have evaluated the presence of G1-S regulatory defects and their clinical relevance in RCC (34, 35). In most malignancies, the detection of cyclin D1 by immunohistochemistry was related to up-regulation of the gene, rapid cell cycling, and poor outcome (45). In contrast, in a small subgroup of ccRCCs the low level of cyclin D1 and p27, a cyclin-dependent kinase inhibitor, was associated with a poor prognosis (34, 35). Our data provided similar results with a risk of metastasis significantly higher in ccRCCs lacking cyclin D1 immunostaining. Furthermore, the subset of cases with tumor cells lacking cyclin D1 but expressing L1 was associated with a higher risk of metastasis. Multivariate analysis confirmed that the L1+/cyclin D1− profile was an independent prognosis factor using pT stage and nuclear grade as other covariables. Although additional studies confirming the validity of this profile as a marker of metastasis in a large cohort are required, the present data strongly suggest that L1 staining with appropriate antibodies should be included in the development of more accurate diagnostic and prognostic methods for ccRCC patients. Additionally, the cell-bound form of L1 could be considered as a potential new target for immunotherapy in ccRCC because antibodies against L1 were shown to inhibit selectively the growth of several tumor cell lines (46).

Interestingly, despite the ectoexpression of L1 also in papRCC, the association with a higher risk of metastasis seems to be characteristic only of ccRCC subtype. This may reflect the existence of distinct genetic aberrations (29) and mechanism of progression between ccRCC and papRCC.

In conclusion, this first follow-up study of L1 expression from earlier stages of renal development to malignancy suggests that L1 ectopic expression may be a key factor of cell homeostasis in the kidney. We showed that L1 is an important prognostic factor in ccRCC and we identified the L1+/cyclin D1− profile as an independent prognostic predictor of metastasis. This profile defines a subset of tumors that may require more aggressive therapies.

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