Subset of Esophageal Adenocarcinoma Expresses Adhesion Molecule L1 in Contrast to Squamous Cell Carcinoma

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Abstract. *Background: Esophageal adenocarcinoma is currently the most rapidly increasing cancer in Western populations. L1 (CD171), a neural cell adhesion molecule, has an essential function in tumor progression and has been shown to be expressed in the proliferating cells of the intestinal crypts in mice. The aim of the current study was to determine L1 expression in esophageal cancer and to evaluate whether L1 could serve as a potential marker and therapeutic target for this tumor type. Materials and Methods: L1 expression was assessed on a tissue microarray with 257 surgically resected esophageal cancer samples by immunohistochemistry with a monoclonal antibody (Clone UJ127). L1 expression was correlated with clinicopathological data. Results: L1 was detected in 22 (9%) of 257 esophageal cases, whereas 235 (91%) were L1 negative. Nineteen (86%) of the 22 L1-positive cases were adenocarcinoma. Cross table analysis showed a significant association between L1 expression and adenocarcinoma subtype ($p<0.001$), but not squamous cell carcinoma. Conclusion: L1 expression in a subgroup of esophageal cancer is specifically prevalent in adenocarcinoma. Data suggest L1 as a potential target for biological therapy in L1-positive esophageal adenocarcinoma patients.*

Esophageal adenocarcinoma is currently the most rapidly increasing cancer in Western populations (1, 2). Esophageal adenocarcinoma and squamous cell cancer are usually detected only at an advanced stage, requiring a multimodal concept for therapy. Despite improvements in its detection, surgical resection and (neo)adjuvant therapy, the overall survival rate of patients with esophageal cancer remains lower than for other solid tumors. Recently, molecular markers have been identified using innovative, molecular technologies to single out predictive and prognostic markers of response to neoadjuvant and adjuvant therapies in esophageal cancer. A variety of techniques have been used to investigate these markers. The most common has been protein expression as evaluated by immunohistochemistry. The goal of the present study was to identify predictive markers to increase the complete pathological response rate in patients treated with neoadjuvant chemotherapy and to identify prognostic markers to select patients for chemotherapy who are at high risk for tumor recurrence after a successful resection. Predictive markers would also benefit patients with metastatic disease to tailor chemotherapy not only for the most effective but also least toxic chemotherapy. Prognostic markers may even be novel targets for new drug developments.

The neural cell adhesion molecule L1 (CD171) is a membrane glycoprotein of the immunoglobulin superfamily that plays a central role in neural development, axonal regeneration and synaptic plasticity in the adult nervous system (3, 4). L1 interacts with a variety of different molecules, such as integrins, and also with itself (5, 6). L1 is not only confined to the nervous system, but it has also been described to play a functional role in the histogenesis of the intestine in mice. L1 is localized in the proliferating epithelial progenitor cells of the intestine, but not in the more differentiated epithelial cells (7, 8). These data suggest that L1 influences tumor progression of intestinal cancer a view that was confirmed in colon cancer (9). In addition, L1 is detected on a variety of tumor cells of neuronal, mesothelial, and epithelial origin, such as neuroblastoma,

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melanoma, lymphoma, small cell lung carcinoma and breast cancer (10-13). Expression profile analysis in multiple human tumors identified L1 as a molecular marker for differential diagnosis and targeted therapy (14-19). Since it is known that A disintegrin and metalloproteinase 10 (ADAM10) cleaves L1 from the tumor cell surface and triggers cell migration (20, 21), L1 in serum is thus a potential diagnostic tool and promises to be a new therapeutic target. The aim of the current study was to determine L1 expression in esophageal cancer.

Materials and Methods

Study design and patients. The study was approved by the Ethics Committee of the Chamber of Physicians in Hamburg, Germany. Written informed consent was obtained from all patients for use of the resected samples. For this study, 116 patients with esophageal adenocarcinoma and 141 patients with squamous cell carcinoma of the esophagus were chosen retrospectively. Patients were selected on the basis of availability of tissues and were not stratified due to rare occurrence and different treatment strategies. All data including sex, histology, depth of tumor invasion, lymph node metastasis, tumor type and disease stage were obtained from the clinical and pathological records.

Tissue microarray (TMA) construction. Tissue samples were fixed in 4% buffered formalin, paraffin embedded, and used for TMA construction as previously described (22). Briefly, hematoxylin-eosin stained sections were made from selected primary tumor blocks (donor blocks) to define representative tumor regions. Tissue cylinders (0.6 mm in diameter) were then punched from that region of the donor block using a home-made semi-automated tissue arrayer. Three μm sections were made by use of the Paraffin Sectioning Aid System (Instrumentics, Hackensack, NJ, USA).

Immunohistochemical staining of L1 and evaluation of expression. Immunohistochemical staining for L1 was performed as described previously (9). Briefly, the heat-induced autoclave antigen retrieval was performed at 120˚C for 5 min with TEC-buffer (pH 7.8). The avidin-biotin-peroxidase method was used for staining (HRP-AEC System, Cell and Tissue Staining Kit; R&D Systems, Minneapolis, MN, USA). Peroxidase blocking (3% H_2O_2 in methanol) lasted 10 minutes. The primary antibody, a murine anti-human L1 monoclonal antibody (IgG_1; Clone UJ127; NeoMarkers, Fremont, CA, USA) binding to the extracellular domain of this molecule, was diluted to 1:50 in Antibody Diluent (Dako, Carpinteria, CA, USA) and slides were incubated overnight in a humidified chamber at 4˚C. For each tissue sample, a section was incubated with irrelevant murine monoclonal IgG1 antibody (MOPC21; Sigma) as a negative control to determine unspecific binding. All washing steps were done with TBS containing 10 g/l Tween 20. Mild counterstaining was performed with hematoxylin for 30 seconds. Samples were considered immunopositive for L1 when >20% of the tumor cells had clear evidence of immunostaining. Peripheral nerves present in nearly all microarrays served as internal positive controls. Immunohistochemical analysis and scoring was performed by three independent investigators (T.R., J.T.K. and U.R.). Two sections were scored differently and in these cases the opinion of the pathologist was decisive.

Statistical analysis. SPSS® for Windows (Version 11.5.1) (SPSS® Inc., Chicago, IL, USA) was used for statistical analysis. The correlation of L1 expression with histological subtype of primary esophageal tumors and histological subtype of primary esophageal lymph node metastases were calculated using a cross table. Statistical analysis was performed with Fisher's test. $P<0.05$ was considered statistically significant.

Results

Characteristics of the patients. A total of 257 surgically treated esophageal cancer patients were included in this study. Out of these, 116 (45%) patients were suffering from esophageal adenocarcinoma and 141 (55%) from squamous cell carcinoma. Briefly, the median age of the study population was 61 years, 203 (79%) patients were male and 54 (21%) female.

Immunohistochemical analysis of L1 in esophageal tumors. L1 expression was determined by immunohistochemical analysis in samples from 116 esophageal adenocarcinoma and 141 squamous cell cancer. Figure 1 shows representative positive staining patterns for L1 of esophageal adenocarcinoma and squamous cell carcinoma. Staining was not detected in normal esophageal tissue. Characteristics of the patients and levels of L1 expression are listed in Table I. Twenty-two (9%) out of 257 esophageal cancer cases were L1-positive. The remaining 235 (91%) patients were negative for L1. Nineteen (86%) out of the 22 patients with L1-positive immunostaining were adenocarcinomas of the esophagus. Only 3 (14%) patients who showed L1-positive staining had a squamous cell carcinoma of the esophagus. Among the 19 L1-positive esophageal adenocarcinomas, 14 (74%) patients with positive lymph node metastases were observed. Altogether, 17 (77%) out of the 22 L1-positive patients had lymph node metastases.

Correlation between L1 expression and esophageal histological subtype. A significant correlation between L1 expression and esophageal adenocarcinoma was found by cross table analysis (Fisher's exact test: $p<0.001$, Table IIa). Nineteen (86%) out of 22 L1-positive patients carried adenocarcinomas of the esophagus. From a total of 116 esophageal adenocarcinomas, L1 was detected in 19 (16%) patients, and 97 (84%) patients were L1 negative. In the esophageal squamous cell carcinoma group, only 3 (2%) patients showed L1 expression, whereas the remaining 138 (98%) were L1 negative. No correlation was observed between survival and L1-positive immunostaining, neither in adenocarcinoma nor in squamous cell carcinoma (data not shown). Table IIb demonstrates L1 expression with histological subtype of primary esophageal lymph node metastases. Eighty-two patients who had esophageal lymph node metastases were included in the study, of which 45

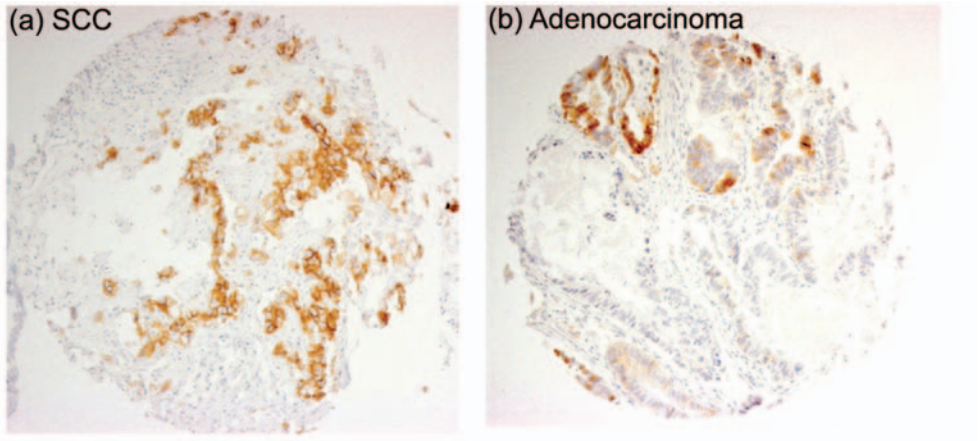

Figure 1. *L1 expression in esophageal cancer. Immunohistochemical staining was performed by the peroxidase method using monoclonal antibody (Clone UJ 127) against L1: (a) represents an L1-positive squamous cell carcinoma (SCC) of the esophagus, whereas (b) shows an L1-positive esophageal adenocarcinoma (Magnification ×200).*

(55%) patients belonged to the adenocarcinoma and 37 (45%) to the squamous cell carcinoma group. Positive L1 expression was shown in 11 (79%) of the adenocarcinomas and in only 3 (21%) of the squamous cell carcinomas with lymph node metastases. The three L1-positive esophageal squamous cell carcinomas were also positive for lymph node metastases.

Discussion

New techniques, such as tissue microarrays, facilitate the standardization of immunohistochemical staining procedures for high numbers of tissue samples and allow pertinent data to be obtained from already embedded tissue.

In an attempt to identify a new tumor marker and therapy target for esophageal cancer, L1 expression was determined on a tissue microarray with 257 surgically resected esophageal cancer specimens. It was found that L1 expression was significantly correlated with the esophageal histological subtype adenocarcinoma, and hardly detected in squamous cell carcinoma of the esophagus. L1 expression in a subgroup of esophageal cancer is specifically prevalent in adenocarcinoma. One limitation of this study was the low number of L1-positive tumors. This low rate of L1 expression might be due to the fact that esophageal adenocarcinoma does not arise from tumors of neural crest or neuroectodermal origin in which L1 is predominantly detected. Although a large number of esophageal cancer patients were included in this study, only in a subset of 16% was L1 expression determined. Rather, it can be assumed that this subset of esophageal adenocarcinomas utilizes L1 as an important factor for metastatic spread and tumor progression as was shown for L1 in a subgroup of colorectal cancer (9).

The cell adhesion molecule L1 (CD171) has emerged as a promising new biomarker for diagnosis and prognosis in multiple human tumors. As a member of the immunoglobulin superfamily, it plays important roles in the development of the nervous system by regulating cell interactions, including neuronal migration, neurite outgrowth and neuron–neuron adhesion (23, 24). Since L1 was described to influence histogenesis of the intestine, detection of L1 expression is frequently examined for differential diagnosis and prediction of prognosis in gastrointestinal tumors (9, 19). L1-positivity was found to be associated with lymph growth (25), micrometastatic spread and poor outcome in human cancer, such as colon cancer and gastrointestinal stromal tumor (26, 27). In contrast to adult tumor entities, where L1 is associated with aggressive clinical behaviour and rather poor prospects, an inverse role of L1 is seen in children with neuroblastoma (28). A trend towards L1-positivity in patients with lymph node metastases was observed in this study which might prove to be significant with larger patient numbers. Furthermore, since the metalloproteinase ADAM10 cleaves L1 from the tumor cell surface and soluble L1 is detected in the serum of tumor patients, an additional diagnostic tool for esophageal cancer patients is available (14, 15). As future prospects, the detection of the tumor marker L1 in serum of esophageal cancer patients would be of high interest. A clear mechanism by which L1 expression contributes to progression of human tumors is still missing. There are also other markers which distinguish between esophageal adenocarcinoma and squamous cell carcinoma. For instance, Her-2, which has been intensely evaluated as a therapeutic target in numerous types of human cancer, was shown to be markedly more frequent in esophageal adenocarcinoma than in squamous cell carcinoma (29).

Table I. *Characteristics of the patients and levels of L1 expression, n (%).*

Variable	L1 expression		*p*-Value
	Negative	Positive	
Total, No. (%)	235 (91)	22 (9)	
Mean age, y±SD	62 ± 10	62 ± 11	
Gender, No. (%)			
Male	184 (72)	19 (7)	
Female	51 (20)	3 (1)	0.352
Tumor type, No. (%)			
Squamous cell carcinoma	138 (54)	3 (1)	
Adenocarcinoma	97 (38)	19 (7)	<0.001
Histological grade, No. (%)			
G1	4 (2)	0 (0)	
G2	132 (51)	8 (3)	
G3	99 (39)	14 (5)	0.139
Tumor depth (pT), No. (%)			
pT1/pT2	99 (39)	15 (6)	
pT3/pT4	136 (53)	7 (3)	0.105
Lymph node involvement (pN), No. (%)			
pN0	81 (32)	5 (2)	
pN1	154 (60)	17 (7)	0.264
Metastasis			
M0	211 (82)	21 (8)	
M1	24 (9)	1 (0.4)	0.706
Residual tumor, No. (%)			
R0	187 (73)	20 (8)	
R1	35 (14)	1 (0.4)	
R2	13 (5)	1 (0.4)	0.388

Table IIa. *Correlation of L1 expression with histological subtype of primary esophageal tumors.*

	L1-negative	L1-positive	Total	*p*-Value
Squamous cell carcinoma	138	3	141	
Adenocarcinoma	97	19	116	
Total	235	22	257	<0.001

Table IIb. *Correlation of L1 expression with histological subtype of primary esophageal lymph node metastases.*

	L1-negative	L1-positive	Total	*p*-Value
Squamous cell carcinoma	34	3	141	
Adenocarcinoma	34	11	116	
Total	68	14	257	0.076

The results of this proof-of-principle study suggest that L1 expression in esophageal tumors is rare and restricted to certain subtypes, but may still be a helpful molecular marker for differential diagnosis and a target for antibody-based therapy in the future.

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