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*RAPID COMMUNICATION*

# L1 is a potential marker for poorly-differentiated pancreatic neuroendocrine carcinoma

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## Abstract

**AIM:** To determine the expression of L1 in pancreatic neuroendocrine tumor and to correlate it with WHO classification of this tumor.

**METHODS**: We retrospectively analyzed L1 expression in 63 cases of pancreatic neuroendocrine tumor by immunohistochemistry on paraffin sections of primary tumors or metastases. Staining was performed by peroxidase technique with monoclonal antibody UJ127.11 against human L1. All tumors were classified according to WHO classification as well-differentiated neuroendocrine tumors and carcinomas or poorly-differentiated neuroendocrine carcinomas.

**RESULTS:** L1 was detected in 5 (7.9%) of 63 pancreatic neuroendocrine tumors. Four (44.4%) of 9 poorly-differentiated carcinomas expressed L1. In contrast, only 1 (1.9%) of 54 well-differentiated tumors or carcinomas was positive for L1. No expression was found in Langerhans islet cells of normal pancreatic tissue. Cross table analysis showed a significant association between L1 expression and classification of neuroendocrine tumors of the pancreas ($P$<0.01).

**CONCLUSION:** L1 is specifically expressed in poorly-differentiated pancreatic neuroendocrine carcinomas that are known to have the worst prognosis. L1 might be a marker for risk prediction of patients diagnosed with pancreatic neuroendocrine carcinomas.



**Key words**: Neuroendocrine pancreatic tumor; Tumor markers; Cell adhesion molecules; L1

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## INTRODUCTION

Neuroendocrine tumors of the gastroenteropancreatic axis are rare and characterized by significant phenotypic differences. They can present themselves as benign or highly malignant and their clinical behavior is very heterogeneous. They are considered to originate from cells of the disseminated neuroendocrine cell system[1]. Most endocrine tumors of the pancreas are well-differentiated neuroendocrine tumors or carcinomas. Frequently, they appear to be malignant with the exception of insulinoma [2]. Fifty percent to sixty percent of these tumors are functionally active and secrete insulin, gastrin, vasoactive intestinal polypeptide (VIP), glucagon or other rare hormones and consequently cause characteristic syndromes. The most important criteria of malignancy include a tumor size of more than 2 cm, angioinvasion and proliferative activity of more than 2% of the tumor cells apart from metastases to the regional lymph nodes and the liver or invasion of adjacent organs [3,4]. Neuroendocrine tumors of the pancreas are classified according to the WHO classification into well-differentiated tumors and carcinomas or poorly-differentiated carcinomas[4,5]. Poorly-differentiated neuroendocrine carcinomas of the pancreas are highly malignant with a bad prognosis[3].

Neoplastic cells frequently re-express adhesion molecules involved in cell migration during tissue morphogenesis and fetal development[6]. The L1 cell adhesion molecule (CD171) is a 200-220 ku type I glycoprotein of the immunoglobulin superfamily and plays a role in development of the nervous system by regulating cell interactions, includ-

ing neuronal migration[7,8]. L1 also mediates neuron-neuron adhesion, neurite outgrowth on Schwann cells, neurite fasciculation and myelination[7]. L1 undergoes homophilic L1-L1 binding and heterophilic interactions with several ligands such as integrins[9,10]. L1 is expressed also in hematopoietic and certain epithelial cells as well as in a variety of tumors, such as of neuroblastomas, melanomas, small cell lung cancer and breast carcinomas[11-15]. Metalloproteinase (ADAM10) also triggers cell migration and cleaves L1 from the tumor cell surface[8,12,16-18]. Recently, it was reported that expression of L1 has a prognostic significance in ovarian and uterine carcinomas and is associated with metastasis of melanomas[19,20]. Furthermore, L1 is expressed in neuroendocrine tumors of the skin[21]. Up-regulation of L1 expression has also been observed in malignant pleural mesotheliomas and malignant peripheral nerve sheath tumors by microarray expression profiling[22,23].

The aim of this study was to determine the expression of L1 in neuroendocrine tumors of the pancreas and its relation to tumor stage of this heterogeneous cancer type. We detected the expression of L1 in 4 (44.4%) of 9 poorly differentiated pancreatic neuroendocrine carcinomas. However, only 1 (1.9%) of 54 well-differentiated tumors or carcinomas was positive for L1. Cross table analysis showed a significant correlation between L1 expression and poorly-differentiated neuroendocrine carcinomas. Our data indicate that L1 is a specific marker for malignant phenotype of pancreatic neuroendocrine carcinomas.

## 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, 63 patients with pancreatic neuroendocrine tumors were chosen retrospectively. We selected patients on the basis of availability of tissues and did not stratify them due to rare occurrence and different treatment strategies. Forty-seven primary tumors of the pancreas and 38 metastases (21 from liver, 16 from lymph nodes and 1 from spleen) were available. All tumors were categorized into 3 groups according to WHO classification of 2 000 into well-differentiated tumors (grade 1a) and carcinomas (grade 1b) or poorly-differentiated neuroendocrine carcinomas (grade 2)[4]. Briefly, this classification was based on tumor size, angioinvasion, proliferating activity, histological differentiation and hormonal activity. 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.

### *Immunohistochemical staining and evaluation of expression*

Immunohistochemical staining was performed for 5-μm thick sections of formalin-fixed and paraffin-embedded tissues placed on pre-coated slides with 3-triethoxysilylpropylamin (Merck, Darmstadt, Germany). After deparaffinization with Rotihistole (Merck) and rehydration in ethanol and TBS (0.05mol/L, pH 7.6) containing 10 g/L Tween 20 (Sigma, Deisenhofen, Germany), tissue sections were pre-treated for 30 min in 10 g/L ammonium chloride ($NH_4Cl$) in TBS, for 15 min in 0.05 mol/L glycine/TBS and then boiled with ChemMate® target retrieval solution (Dako, Hamburg, Germany) in a microwave oven according to the manufacturer´s instructions. Staining was performed with the peroxidase method (HRP-AEC System, Cell and Tissue Staining Kit; R&D Systems, Minneapolis, MN, USA). 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 at 1:50 in antibody diluent (Dako) and slides were incubated overnight in a humidity chamber at 4 °C [24]. For each sample one slide, a control section was incubated with irrelevant murine monoclonal $IgG_1$ (MOPC21; Sigma) as a negative control to determine the unspecific binding. All washing steps were done with TBS containing 10 g/L Tween 20. Counterstaining was performed with Haemalaun Mayer (Merck) for 30 s followed by Mayer´s haematoxylin solution (Merck) for 7 min. At last, slides were covered with coverslips with aqueous mounting medium (Aquatex®; Merck). Specimens were considered immunopositive for L1 when >20% of the tumor cells had clear evidence of immunostaining. Peripheral nerves present in almost all sections served as internal positive controls. Langerhans islet cells were negative for L1 in normal pancreatic tissue. Immunohistochemical analysis and scoring of the sections were performed by two independent investigators and one pathologist in a blinded fashion. Two sections were scored differently and in these cases the opinion of the pathologist was decisive.

### *Statistical analysis*

We used SPSS for Windows (SPSS Inc., Chicago, IL USA) for statistical analysis. The immunostaining results of L1 and WHO classification of neuroendocrine tumors of the pancreas were calculated using a cross table and statistical analysis was performed with *F*-test. $P<0.05$ was considered statistically significant.

## RESULTS

### *Characteristics of the patients*

Sixty-three patients suffering from pancreatic neuroendocrine tumor were included in the study. Characteristics of the patients are listed in Table 1. Briefly, the median age of the study population was 57 years, 32 (50.8%) patients were male and 31 (49.2%) female. According to WHO classification for neuroendocrine tumors of the gastroenteropancreatic axis, 50 (79.4%) were classified as well-differentiated neuroendocrine tumors (grade 1a), 4 (6.3%) as well-differentiated carcinomas (grade 1b) and 9 (14.3%) as poorly-differentiated neuroendocrine carcinomas (grade 2), being the most malignant phenotype. Eleven (17.5%) tumors showed hormone production and 6 (9.5%) of 63 patients suffered from endocrine neoplasia (MEN) type I.

### *Immunohistochemical analysis of L1 in pancreatic neuroendocrine tumors*

L1 expression was determined by immunohistochemical analysis in samples from 63 pancreatic neuroendocrine tu-

**Table 1 Characteristics of the patients and levels of L1 expression *n*(%)**

| Variable | Patients | L1-positive tumors |
|---|---|---|
| *Total* | 63 | 5 (7.9) |
| Male | 32 (50.8) | 4 (12.5) |
| Female | 31 (49.2) | 1 (3.2) |
| *WHO classification of neuroendocrine pancreatic tumour* | | |
| Well-differentiated neuroendocrine tumour (grade 1a) | 50 (79.4) | 1 (2.0) |
| Well-differentiated neuroendocrine carcinoma (grade 1b) | 4 (6.3) | 0 |
| Poorly-differentiated neuroendocrine carcinoma (grade 2) | 9 (14.3) | 4 (44.4) |
| *Hormone production* | | |
| Yes | 11 (17.5) | 0 |
| No | 52 (82.5) | 5 (9.6) |
| *Multiple endocrine neoplasia (MEN)-I* | | |
| MEN-I | 6 (9.5) | 0 |
| No MEN-I | 57 (90.5) | 5 (8.8) |

**Table 2 Correlation of L1 expression with WHO classification**

| WHO classification | L1-negative | L1-positive | Total |
|---|---|---|---|
| Well-differentiated neuroendocrine tumors and Well-differentiated neuroendocrine carcinomas (grade 1a and 1b) | 53 | 1 | 54 |
| Poorly-differentiated neuroendocrine carcinomas (grade 2) | 5 | 4 | 9 |
| Total | 58 | 5 | 63 |

$P<0.01$ by Fisher's test (two-sided)

mor patients. Forty-seven primary tumors of the pancreas and 38 metastases (21 from liver, 16 from lymph nodes and 1 from spleen) were available and immunostained. In 18 patients, both primary tumor and metastases (12 from lymph nodes, 3 from liver, 2 from both lymph nodes and liver, 1 from liver and spleen) were investigated. In 16 patients, only metastases were available (14 from liver, 1 from lymph nodes, 1 from both lymph nodes and liver). No differences in terms of positivity or negativity of L1 expression were detected between primary tumor and metastases in any patient. Figure 1 shows the representative negative and positive staining patterns for L1 of pancreatic neuroendocrine tumors. Staining was not detected in normal pancreatic islet cells.

Five (7.9%) of 63 cases were L1-positive (Table 1). were stained. The remaining 58 (92.1%) patients were negative for L1. According to the WHO classification, 4 (44.4%) of 9 poorly-differentiated neuroendocrine carcinomas (grade 2) were L1-positive. Only 1 (1.9%) of 54 well-differentiated neuroendocrine tumor samples (grade 1a) was L1-positive. None of the 4 well-differentiated neuroendocrine carcinomas (grade 1b) was positive for L1. Although only 9 poorly-differentiated pancreatic neuroendocrine carcinomas (grade 2) were available, these results showed that L1 was specifically expressed in poorly- differentiated tumors. Forty-four point four percent of these most highly malignant tumors were positive for L1 compared to 1.9% in the group of well-differentiated neuroendocrine tumors or carcinomas (grade 1a and 1b).

### *Correlation between L1 expression and WHO classification of tumor*

A significant correlation between L1 expression and well-differentiated neuroendocrine tumor (grade 1a) and (grade 1b) or poorly-differentiated neuroendocrine carcinoma (grade 2) was found by Fisher's exact test ($P<0.01$, Table 2).

## DISCUSSION

Pancreatic neuroendocrine tumors or carcinomas are rare and clinically very heterogeneous. Neuroendocrine-specific molecules are positive markers of endocrine differentiation in tumor cells[1]. Little is known about the molecular differences between benign and malignant phenotypes of neuroendocrine tumors of the pancreas. Cell adhesion molecules, such as L1, have been repeatedly implicated in tumor progression and metastasis. In this study, we determined L1 expression in 7.9% of 63 cases of pancreatic neuroendocrine tumor. Since L1 is not expressed in normal Langerhans islet cells, which are believed to be the precursor cells of pancreatic neuroendocrine tumors, an up-regulation of L1 expression in tumor cells may be associated with tumorigenesis in this tumor type.

We used immunohistochemical analysis for detection of L1 in tumor cells. Because the optimal cutpoint approach has some limitations in statistical evaluation of prognostic factors, we chose a cutpoint of 20% L1-positive tumor cells in the analysed cell population, achieving an easy discrimination of immunostained tumor tissue as proposed by Altman and colleagues[25].

In our study, there were 9 patients with poorly-differentiated neuroendocrine carcinoma according to WHO classification (grade 2). Out of these, 4(44.4%) had positive L1 expression. L1 In contrast, only 1 (1.9%) of 54 well-differentiated tumors and carcinomas (grade 1a and 1b) was positive for L1. Statistical analysis showed a significant correlation between L1 expression and poor differentiation of pancreatic neuroendocrine tumors (grade 2), suggesting that L1 is a marker for poorly differentiated and highly malignant pancreatic neuroendocrine carcinomas. Although the number of L1-positive tumors in our study was too low for a final conclusion, the number of L1-negative tumors supports our notion that L1 is a marker for malignancy of this tumor entity.

These observations are in agreement with previous studies correlating expression of L1 in different tumors of neuroectodermal origin, such as melanomas or uterine and ovarian carcinomas, with malignancy and poor prognosis[15,19-21,26]. Our results prove that expression of L1 can also be found in another neuroendocrine tumor, namely the neuroendocrine tumor of the pancreas.

Further studies are needed to determine the potential prognostic value of L1 expression in patients suffering from pancreatic neuroendocrine carcinomas. Our data also indicate that downregulation of L1 expression by antisense technologies may be used as a therapeutic method.

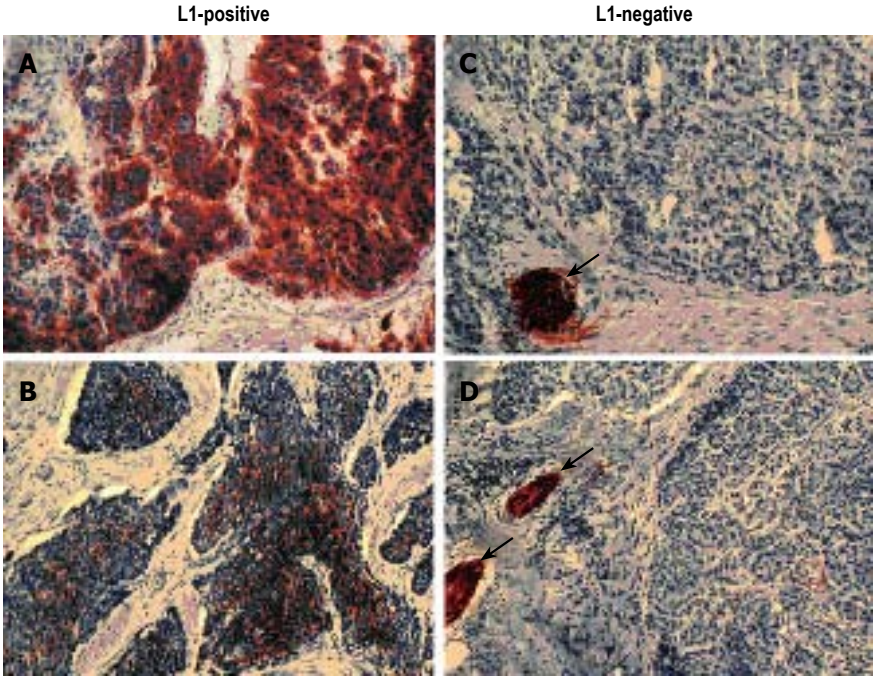


**Figure 1** L1 expression in pancreatic neuroendocrine tumours or carcinomas. Immunohistochemical staining was performed by peroxidase method using monoclonal antibody UJ.127 against L1. Poorly-differentiated L1-positive pancreatic neuroendocrine carcinomas (grade 2; A and B) were shown in comparison to well-differentiated L1-negative tumours (grade 1a; C and D). Peripheral nerves (*arrows*) stained in (C, D) served as internal positive controls (Magnification ×200 (A and C) and ×400 (B and C)).

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