Evaluation of L1CAM, SOX11, and chromogranin expression in pancreatic tumors: an immunohistochemical study

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Received: 31-Jul-2024
Revised: 09-Aug-2024
Accepted: 20-Aug-2024
Published: 21-Feb-2025

Egyptian Journal of Pathology 2024, 44:102–110

Introduction
Pancreatic duct adenocarcinoma (PDAC) is the 11th most common type of cancer and its incidence and death rates are steadily rising. In contrast to PDAC and pancreatic neuroendocrine tumors (PNETs), solid pseudopapillary neoplasm (SPN) is a low-grade malignant pancreatic tumor that exhibits distinct characteristics in terms of tumor aggressiveness, treatment, and prognosis.
Aim
To investigate the expression of L1CAM, SOX11, and chromogranin in PDAC, SPN, and PNETs and to show their diagnostic and prognostic significance.
Materials and methods
Retrospective Immunohistochemical staining of L1CAM, Sox11, and chromogranin was performed on selected 54 cases of pancreatic tumors.
Results
L1CAM was highly expressed in 73.3% of PDAC cases compared with 81.8% of SPN and 100% of PNET cases. SOX11 was positive in 90.9% of SPN, but negative in 100% of PDAC and 93.3% of PNETS cases. Chromogranin was positive in 76.9% of PNETS. SOX11 is a highly sensitive (100%) marker for discriminating between SPN and PDAC. Both SOX11 and chromogranin are highly specific (100%) and sensitive (90.9%) markers in differentiating SPN from PNET. L1CAM was significantly positively correlated with tumor grade (P=0.02), T stage (P=0.02), lymph node metastasis (P=0.002), LVI (P=0.000), and distant metastasis (P=0.046) of PDAC studied cases.
Conclusion
SOX11 could be considered a highly sensitive marker for differentiating SPN from PDAC and PNETs. The combined expression of L1CAM, SOX11, and chromogranin may play a valuable role in solving this diagnostic challenge. L1CAM might have prognostic significance for PDAC and, hence, target therapy.

Keywords:
L1CAM, pancreatic tumors, pancreatic neuroendocrine tumors, SOX11, solid pseudopapillary neoplasm

Egypt J Pathol 2024, 44:102–110

1687-4277

Introduction

According to the Global Cancer Observatory (2020), pancreatic duct adenocarcinoma (PDACs) ranks as the second most common cause of cancer-related death in the United States of America, despite being the 11th most common malignancy. The rate of both incidence and death is constantly increasing (Rahib *et al.*, 2014).

Most cases of PDAC are discovered at an advanced stage with widespread metastases. Consequently, at the time of diagnosis, there is no feasible alternative for a curative surgical resection for the majority of patients. Moreover, PDAC cells have innate or acquired resistance to chemotherapy medicines which are typically ineffective and frequently linked to a poor prognosis (Sebens Müerköster *et al.*, 2007). Therefore, to enhance diagnostic processes and treatment alternatives, it is imperative to identify novel molecular tumor markers of PDAC.

Solid pseudopapillary neoplasm (SPN) is a low-grade malignant pancreatic tumor of uncertain histogenesis, as described by Kloppel *et al.* (2010). Cellular differentiation, genomic, transcriptomic, and proteomic profiles of SPN differ from PDACs and neuroendocrine neoplasms, as noted by Guo *et al.* (2017).

Neuroendocrine tumors (NETs) and SPNs of the pancreas differ significantly in terms of tumor aggressiveness, treatment, and prognosis. NETs are considered to have malignant potential, with 40–90% showing gross invasive growth or metastasis, according to sources (Mansour and Chen, 2004), whereas SPNs

have low-grade malignancy, exhibiting local invasion or metastasis in only 20% of the cases.

Consequently, the 5-year survival rate for patients with neuroendocrine tumors (NETs) is lower than that of patients with SPNs, with 65% for NETs and 95% for SPNs (Hochwald *et al.*, 2002). Surgical resection is the primary treatment option for both localized and metastatic tumors.

Due to these differences in clinical features and treatment strategies, it is essential to carefully differentiate between NETs and SPNs, as this can significantly impact patient outcomes. Besides morphological evaluation, immunohistochemical analysis plays a crucial role in distinguishing between these two tumor types, particularly when there is limited material obtained through endoscopic ultrasound-guided fine-needle aspiration (Hewitt *et al.*, 2012).

L1 cell adhesion molecule (L1CAM) is a 200–220-kD glycoprotein and a member of the immunoglobulin superfamily. It is frequently overexpressed in various tumors, induces tumor migration, and often serves as an unfavorable prognostic factor (Hua *et al.*, 2016).

SRY-related HMG-box (SOX) proteins are transcriptional regulators involved in diverse tissues and developmental processes. The mammalian SOX family consists of 20 proteins functionally categorized into nine subgroups (Julian *et al.*, 2017). SOX11 is a member of the SOX gene family, which is frequently deregulated in various tumor types and can act as a tumor suppressor or oncogene (Li *et al.*, 2015).

Chromogranin A is a marker for neuroendocrine secretory granules and belongs to the family of acidic proteins that constitute a major component of secretory granules in various endocrine and neuroendocrine cells (Tiemann *et al.*, 2006).

The objective of this research is to evaluate the diagnostic significance of the immunohistochemical expression of L1CAM, SOX11, and chromogranin A in pancreatic neuroendocrine tumors (PNETs), PDAC, and solid pseudopapillary tumors (SPN) and correlating their expression with various clinicopathological features. This will help determine their role in the prognosis of pancreatic carcinoma and inform targeted therapy.

Materials and methods

This is a selected controlled retrospective study that included formalin-fixed paraffin-embedded blocks of 54 selected cases of pancreatic tumors designed as 30 cases of pancreatic duct carcinoma, 11 cases of SPN, and 13 cases of PNETs. Thirty-six cases were obtained by surgical resection (Whipple operations or distal pancreatectomy) and 18 cases by endoscopic ultrasound-guided fine-needle aspiration biopsy. Ten cases of chronic pancreatitis were used as control. Blocks were obtained from archives of the Pathology Department and Early Cancer Detection Unit (ECDU), Faculty of Medicine, Benha University, from March 2013 to July 2023). The study was approved by the ethics committee of the Faculty of Medicine, Benha University (RC 23-1-2024).

Paraffin-embedded tissue sections were prepared from the obtained specimens. To confirm diagnosis and grading, two pathologists reviewed hematoxylin and eosin sections. Cases of PDAC were graded according to glandular differentiation into well, moderate, and poorly differentiated carcinoma (Ducreux *et al.*, 2015). Staging was done according to the 8th edition AJCC Cancer Staging Manual 2017 (Amin *et al.*, 2017).

Only cases with available blocks and clinical pathology data were included in this analysis. However, cases with a previous history of chemotherapy or without clinical pathological information were excluded from the current study.

Immunohistochemical study

Three formalin-fixed, paraffin-embedded, 4-μm tissue sections were prepared on a positively charged slide. For each case, immunohistochemical detection for L1CAM, SOX11, and chromogranin A was done. The streptavidin–biotin procedure was used in accordance with the guidelines provided by the manufacturer (NeoMarker, LabVision, California,, USA). Sections were visualized with 0.02% diaminobenzidine solution. Finally, sections were counterstained with hematoxylin and then dehydrated and mounted. A negative control was used for each marker by omitting the primary antibody as shown in Table 1.

Immunohistochemical interpretation

Bergmann *et al.* (2010) provided guidelines for evaluating the L1CAM immunohistochemistry staining, stating that the tumor cell is regarded as positive if 10% or more exhibit moderate to strong membranous staining. According to Dinarvand *et al.* (2022) for SOX11, a minimum of nuclear staining in more than 10% of tumor cells was deemed positive. Finally, the assessment of chromogranin A's cytoplasmic positivity is determined by Ohara *et al.* (2016).

Statistical analysis

Statistical analysis was conducted using the SPSS (version 26 for Windows) software package, following

Table 1 Data for using L1CAM, SOX11, and chromogranin

Antibody	Type	Cat. No.	Dilution	Positive control	incubation	Antigen retrieval
L1CAM	Mouse Monoclonal antibody	Thermo Fisher Scientific Catalog # MA5-14140	1: 100	Nerve tissue	15 min	Citrate buffer pH 7.
SOX11	Rabbit Monoclonal antibody	Cat. No. 6664-RBM5-P0	1: 100	Mantle cell lymhoma	30 min	Citrate buffer PH 6.0
Chromogranin	Rabbit Polyclonal antibody	Thermo Fisher Scientific, Cat. No MA5-13096	1: 100	Normal adrenal tissue	30 min	Citrate buffer pH 7.4

L1CAM, L1-cell adhesion molecule; SOX11, SRY-related HMG.

the guidelines of Spearman's correlation coefficient. The correlation between several variables was determined using Fisher's exact test. A *P* value of less than 0.05 was considered statistically significant, while a *P* value of less than 0.01 was deemed highly significant. Receiver-operating characteristic curves were constructed to assess the effectiveness of L1CAM, SOX11, and chromogranin A in distinguishing between SPN, PNET, and PDAC.

Results

Clinicopathological features of the studied cases

This study included 54 cases of pancreatic tumors designated as 30 cases of pancreatic duct carcinoma, 11 cases of SPN, 13 cases of PNETs, and 11 cases of SPN. The clinicopathological variables of the studied PDAC cases are listed in Table 4.

Immunohistochemical results

Comparison of L1CAM, SOX11, and chromogranin expression among the studied groups

L1CAM was highly expressed in 22 (73.3%) cases of PDAC as shown in Fig. 1c, while nine (81.8%) cases of SPN in Fig. 1a and all cases of PNET in Fig. 1a, b showed low expression with statistically significant correlation (*P*=0.000).

SOX11 was positive in 10 (90.9%) cases of SPN as shown in Fig. 1d, negative in all cases of PDAC (Fig. 1e), and 12 (92.3%) cases of PNETs in Fig. 1f with a highly statistically significant difference (*P*<0.01).

Chromogranin was positively expressed in 10 (76.9%) cases of PNETs (Fig. 1h), while it was negative in all cases of PDAC and SPN with a highly statistically significant difference (*P*<0.01) as shown in Table 2 and Fig. 1g, i.

Diagnostic performance of L1CAM, SOX11, and chromogranin in discriminating SPN from PNET and PDAC

L1CAM is a specific marker in discriminating PDAC from SPN (82% specificity and 73.3% sensitivity), while it showed very low sensitivity in differentiating SPN versus PNETs (100% specificity and 18.2% sensitivity).

SOX11 is a highly sensitive marker in a setting of differentiation between SPN and PDAC (100% sensitivity and 90% specificity). Moreover, both SOX11 and chromogranin are highly specific and sensitive markers in differentiating SPN from PNET (93.9% specificity, 90% sensitivity, and 100% specificity and 76.9% sensitivity, respectively) as shown in Table 3.

The combined expression of L1CAM, SOX11, and chromogranin has high accuracy in discriminating SPN from PNET (100% specificity, 18.2% sensitivity) as shown in Fig. 2, while a combined expression of both SOX11 and chromogranin was more and highly sensitive (100% specificity, 90.9% sensitivity) in distinguishing SPN from PNET as shown in Table 3, Fig. 2.

Correlation between the studied markers L1CAM, SOX11, and chromogranin

There was no significant correlation between L1CAM, SOX11, or either SOX11 and chromogranin among the studied groups (*r*=0.106, *r*=0.464). There was a statistically significant inverse correlation between L1CAM and chromogranin among the studied cases (*r*=004).

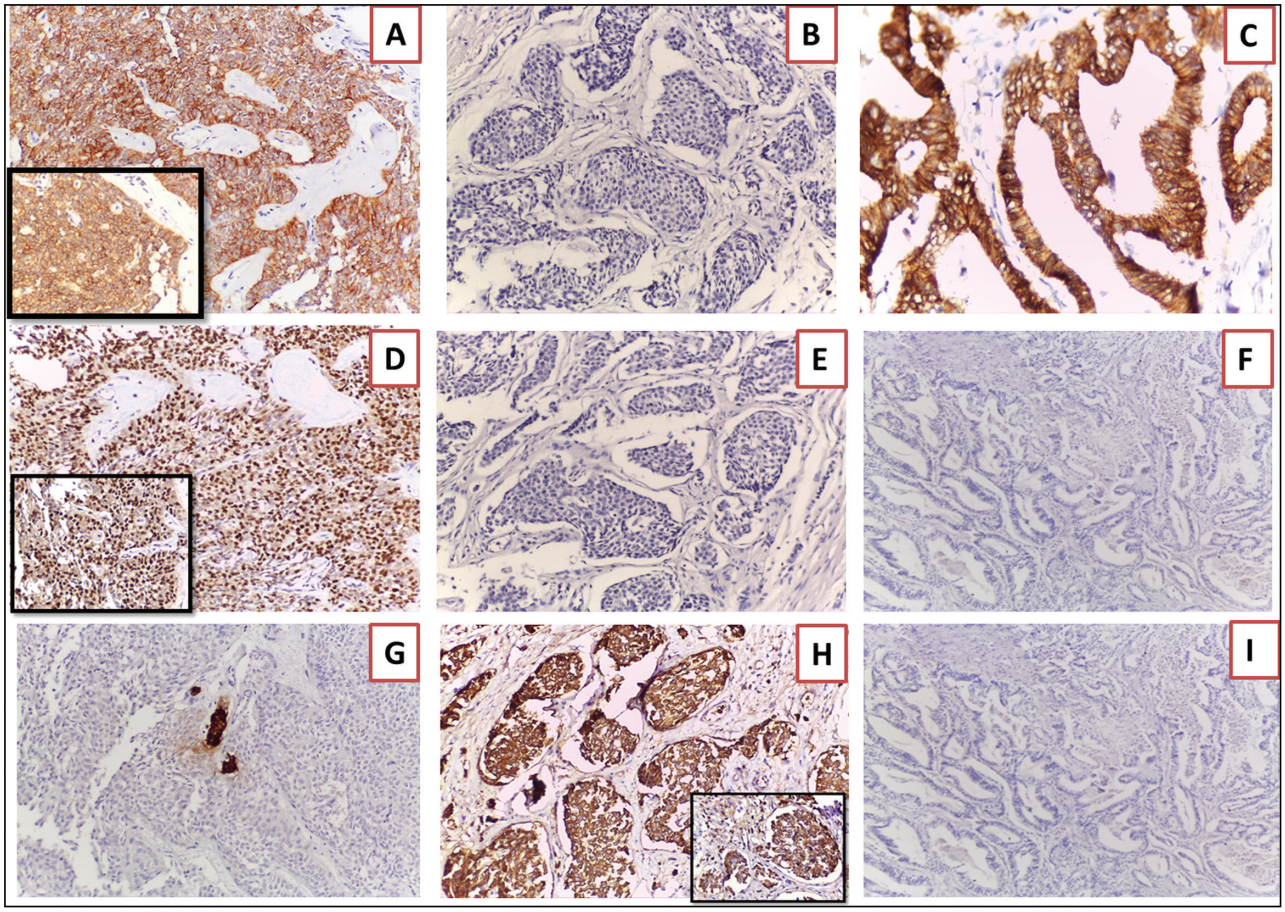
Relation between L1CAM expression and clinicopathological parameters in the studied cases of PDAC

There was a statistically significant positive correlation between L1CAM expression and higher grade (*P*=0.02) as shown in Fig. 3b, advanced T stage (*P*=0.02), positive lymph node metastasis (*P*=0.002), positive LVI (*P*=0.000), and distant metastasis (*P*=0.046) as shown in (Table 4).

Discussion

SPN is a low-grade malignancy, and its treatment usually results in a cure rate of more than 95% (Cai *et al.*, 2014). It is crucial to differentiate SPN from PNETs or PDAC because they require distinct treatment approaches. This retrospective controlled study included 54 cases of pancreatic tumors, including

Figure 1



L1CAM, SOX11, and chromogranin IHC expression among the studied groups. (a) Diffuse strong cytoplasmic/membranous expression of LICAM in SPN (IHC, ×200, inset ×400). (b) Negative expression of LICAM in PNET. (c) Strong expression in PDAC (IHC, ×400). (d) Positive nuclear expression of SOX11 in SPN (IHC, ×200, inset ×400) while PNET. (e), PDAC (f) are negative for SOX11. (h) Diffuse granular cytoplasmic expression of chromogranin in PNET (×200, IHC, inset ×400) while negative expression in SPN (g), PDAC (i). PDAC, pancreatic duct adenocarcinoma; PNETs, pancreatic neuroendocrine tumors; SPN, solid pseudopapillary neoplasm.

Table 2 Comparison of L1CAM, SOX11, and chromogranin among the studied cases

Type Marker	SPN (*N*=11) [*n* (%)]	PNET (*N*=13) [*n* (%)]	PDAC (*N*=30) [*n* (%)]	*P* value
L1CAM				
Low	9 (81.8)	13 (100.0)	8 (26.7)	0.000**
High	2 (18.2)	0	22 (73.3)	
SOX11				
Negative	1 (9.1)	12 (92.3)	30 (100.0)	0.000**
Positive	10 (90.9)	1 (7.7)	0	
CG				
Negative	11 (100.0)	3 (23.1)	30 (100.0)	0.000**
Positive	0	10 (76.9)	0	

CG, chromogranin; L1CAM, L1-cell adhesion molecule; PDAC, pancreatic ductal adenocarcinoma; PNETs, pancreatic neuroendocrine tumors; SOX11, SRY-related HMG-box; SPN, solid pseudopapillary neoplasm.
**Highly significant.

30 cases of PDAC, 13 cases of PNETs, and 11 cases of SPN.

This study evaluated the immunohistochemical expression of L1CAM, SOX11, and chromogranin in all cases.

In this study, there was a highly significant difference in the immunohistochemical expression of L1CAM between SPN and PNETs compared with PDAC (P<0.01). L1CAM was highly expressed in 73.3% of PDAC cases and 81.8% of SPN cases, and all PNET cases showed low or negative expression.

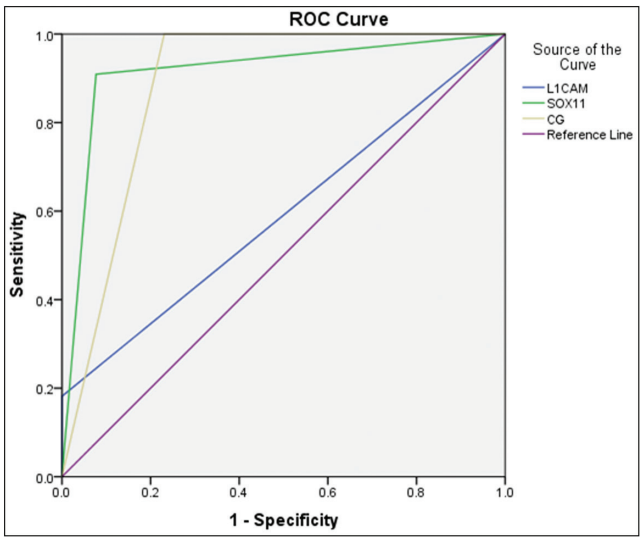
These findings are supported by previous studies, including one conducted by Bergmann *et al.* (2010), who found L1CAM expression in 92.7% of primary PDACs, and another by Ben *et al.* (2010), who found

Table 3 Diagnostic performance of L1CAM, SOX11, and chromogranin in solid pseudopapillary pancreatic neoplasm versus PNETs and PDAC

Markers	SPN vs. PNETs		SPN vs. PDAC	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
L1CAM	18.2	100	73.3	82
SOX11	90.9	93	100	90.9
CG	100	76.9	0.0	10
SOX11+ve/L1CAM+ve	18.2	100	0.0	82
SOX11+ve/CG-ve	90.9	100	0.0	10
SOX11+ve/L1CAM +VE//CG –ve	18.2	100	0.0	81.8

CG, chromogranin; L1CAM, L1 cell adhesion molecule; PDAC, pancreatic ductal adenocarcinoma; PNETs, pancreatic neuroendocrine tumors; SOX, SRY-related HMG-box; SPN, solid pseudopapillary neoplasm.

Figure 2



Diagnostic performance of L1CAM, SOX11, and CG markers in discriminating SPN versus PNET. PNETs, pancreatic neuroendocrine tumors; SPN, solid pseudopapillary neoplasm.

positive immunostaining for L1CAM in 60.6% of PDAC cases. Similarly, a study by Liszka (2021) found that all cases of SPN in a training cohort showed L1CAM expression (usually weak, median extent 45% of cells), whereas 90% of the studied cases in a validation cohort (SPN) were L1CAM-positive (usually weak expression, median extent 15% of cells), and among NET cases, L1CAM was found in only two (7%) cases.

In a study conducted by Inaguma *et al.* (2016), L1CAM expression was found to be present in the majority of PNET, but the frequency and intensity of staining varied. Specifically, a weak and strong expression was observed in 50 and 23.8% of the cases, respectively.

Conversely, Rawnaq *et al.* (2012) showed strong L1CAM expression in only 8% of 63 pancreatic neuroendocrine carcinomas examined. Kaifi *et al.* (2006) also found L1CAM expression in only 1.9% of 54 well-differentiated PNETs analyzed. However, Huszar *et al.* (2006) did not detect L1CAM expression in any of the 10 neuroendocrine pancreatic carcinomas.

Discrepancies in the prevalence of L1CAM expression may be attributed to various factors, such as experimental conditions, scoring criteria, clone of the antibody used in the analysis, and population bias, as suggested by Bergmann *et al.* (2010).

The L1 cell adhesion molecule, also known as neural cell (NC) adhesion molecule L1 (L1CAM, N-CAM L1, CAML1, CD171, and MIC5), is crucial for several aspects of neural development, including neuronal adhesion and migration, axonal guidance, synaptogenesis, neurite growth and fasciculation, myelination, and cell survival as demonstrated in a systematic review by van der Maten *et al.* (2019).

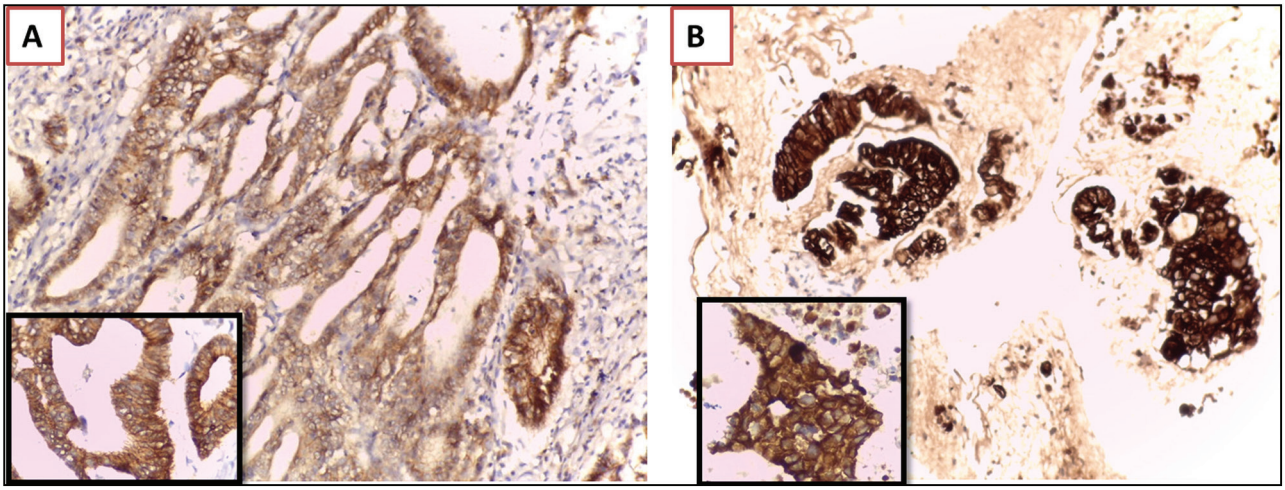
The NC is a group of migratory embryonic neuroectodermal cells, which gives rise to various types of tissues throughout the body. Research has identified similarities between NC cell migration and cancer progression (Wislet *et al.*, 2018).

It was proposed that SPN may originate from NC cells. This hypothesis was based on the assessment of transcriptomic and proteomic/IHC SPN profile (Ye *et al.*, 2012).

L1CAM serves as a marker for NC cell migration into the gut, a process that was first identified by Anderson *et al.* (2006). In addition, L1CAM expression is frequently observed in neoplasms originating from the NC/neuroectoderm, including neuroblastomas, granular cell tumors, phaeochromocytomas, schwannomas, primitive neuroectodermal tumors, and paragangliomas. According to Liszka (2021), up to 100% of these entities exhibit L1CAM expression.

L1CAM is identified as a target gene of the β-catenin/Wnt signaling pathway, which may play a role in the development of tumors for SPN (Gavert *et al.*, 2005).

Figure 3



L1CAM expression in PDAC. (a) PDAC, grade 2 showed lower membranous expression while the high grade one obtained by EUS-FNAC (b) showed an expression (IHC, ×200, inset ×400). EUS-FNAC, endoscopic ultrasound-guided fine-needle aspiration cytology; PDAC, pancreatic duct adenocarcinoma.

Table 4 Relation of L1CAM expression and clinicopathological parameters in the studied cases of PDAC

Variables	L1CAM	Low (*N*=8) [*n* (%)]	High (*N*=22) [*n* (%)]	χ^2 test	*P* value
Age	≤60 (18)	4 (50.0)	14 (63.6)	0.455	0.55
	>60 (12)	4 (50.0)	8 (36.4)		
Sex	Male (17)	4 (50.0)	13 (59.1)	0.197	0.657
	Female (13)	4 (50.0)	9 (40.9)		
Grade	Grade I+II (20)	8 (40.0)	12 (60.0)	5.455	0.02*
	Grade III (10)	0	10 (100.0)		
T stage	T1+T2 (10)	0	10 (45.5)	5.45	0.02*
	T3+T4 (20)	8 (100.0)	12 (54.5)		
	Stage III+IV (13)	3 (37.5)	10 (45.5)		
LVI	+ve (20)	1 (5.0)	19 (95.0)	14.403	0.000**
	–ve (10)	7 (70.0)	3 (30.0)		
LNM	N0 (16)	8 (50.0)	8 (50.0)	9.54	0.002**
	N1 (14)	0	14 (100.0)		
Distant metastasis	M0 (22)	8 (36.4)	14 (63.6)	3.967	0.046**
	M1 (8)	0	8 (100.0)		
TNM stage	Stage I+II (17)	5 (62.5)	12 (54.5)	0.151	0.697
	Stage III+IV (13)	3 (37.5)	10 (45.5)		
Perineural invasion	Positive (11)	3 (37.5)	8 (36.4)	0.003	0.954
	Negative (19)	5 (62.5)	14 (63.6)		

L1CAM, L1-cell adhesion molecule; LNM, lymph node metastasis; LVI, lympho-vascular invasion.
*Significant.
**Highly Significant.

LEF1, a marker for these tumors (Kim *et al.*, 2017), binds to L1CAM to promote its expression (Gavert *et al.*, 2008).

Concerning the expression of L1CAM among the examined groups, it serves as a precise marker for distinguishing between PDAC and SPN, achieving 82% specificity and 73.7% sensitivity.

However, in differentiating between SPN and PNETs, its sensitivity is quite low at 18.2%. These results align with the findings of Bergmann *et al.* (2010) and Liszka (2021), the former of which reported that L1CAM was generally detected in PDACs, while the latter demonstrated that weak L1CAM expression was a nearly universal characteristic of SPN. This limitation may reduce the potential usefulness of this stain for SPN diagnosis.

In the current investigation, a statistically significant difference in the IHC expression of SOX11 was observed among the various study groups ($P<0.001$). In the study, SOX11 was expressed in 90.9% of SPN, 100% of PDAC, and 92.3% of PNET. These results are consistent with those of Cao *et al.* (2023), who reported positive signals for SOX11 in the tumor nucleus with

a positive expression rate of 92.86% in SPN, but only 8.93% in PNET. Furthermore, SOX11 was absent in all cases of PDAC.

According to Wang *et al.* (2020), SOX11 was found to be positive in 92.1% of SPN cases, but negative in all studied cases of PNETs and PDAC cases. This finding aligns with the results of Harrison *et al.* (2018), who detected nuclear immunoreactivity for SOX11 in all SPN cases and 16% of the control tumors (four neuroendocrine tumors and one pancreatoblastoma).

Foo *et al.* (2017) also reported nuclear reactivity for SOX11 in all studied cases of SPNs and 0 out of 13 non-SPNs (PNET and acinar cell carcinoma) in fine needle cytology specimens. Cho *et al.* (2008) reported SOX11 nuclear immunoreactivity in 28 (82%) of 34 SPN cases.

In the present study, SOX11 demonstrated high sensitivity in differentiating SPN from PNETs, with 90.9% sensitivity and 93% specificity, and from PDAC, with 100% sensitivity and 90.9% specificity.

These results align with those of Wang *et al.* (2020), which found the specificity and sensitivity of SOX11 in SPN to be 92.1 and 100.0%, respectively.

Harrison *et al.* (2018) also reported that SOX11 was 100% sensitive and 84% specific in the diagnosis of SPN. Furthermore, Foo *et al.* (2017) demonstrated in their study that SOX11 was 100% specific and sensitive in the diagnosis of SPN.

Moreover, Li *et al.* (2015) discovered a strategy to identify candidate biomarkers for differentiating SPN from PNETs and PDAC. They found SPN-relevant genes, including seven transcription factors such as SOX11, SMAD3, and SOX4, which could correctly differentiate SPN from PNETs.

SPN is a tumor with unclear cell origin and direction of differentiation. Although some studies have suggested that aberrant Wnt/β-catenin signaling may drive tumorigenesis, relatively few genetic alterations have been detected in SPNs compared with other types of pancreatic tumors. Approximately 90% of SPNs harbor gain-of-function mutations in CTNNB1 (Springer *et al.*, 2015).

However, gene expression studies at the mRNA level have revealed a larger number of differentially expressed genes and signaling pathways in SPNs, indicating more complex underlying molecular mechanisms in the pathogenesis of SPN (Li *et al.*, 2015).

While SOX11 is strongly expressed in mantle cell lymphoma, medulloblastoma, and malignant glioblastoma, and has prognostic value in epithelial ovarian tumors and high-grade breast cancer, other members of the SOX protein family, such as SOX15, SOX7, SOX17, SOX6, and SOX9, act as tumor suppressors and are opposed to the Wnt/β-catenin signaling pathway in a variety of malignant tumor types (Bernard and Harley, 2010; Zhao *et al.*, 2016), and do not express as much.

Gene regulatory network analysis was used by Li *et al.* (2015) to find candidate genes that had the shortest routes from 26 known SPN-related genes, such as CDH1, TCF/lymphoid enhancer-binding factor 1 (LEF1), CTNND1, and CTNNB1. The findings demonstrated that SOX11 mRNA was substantially elevated in SPNs (5.62-fold) compared with normal pancreas and 5.02-fold compared with PNETs. Nevertheless, it is still unclear exactly what biological role SOX11 plays in SPN.

By directly interacting with molecules like TCF and β-catenin, several additional SOX factors also modify cell signaling pathways (Moradi *et al.*, 2017). The SOX-HMG signature amino acid sequence, which is the defining structure for SOX proteins to mediate binding to DNA consensus motifs, may allow SOX11 to directly bind to other transcription factors and play a regulatory role in SPN. The finding that SOX11 directly interacts with cyclin D1 during the early stages of the development of mantle cell lymphoma lends credence to this theory (Beà and Amador, 2017).

In this study, chromogranin A (CgA) was expressed in 76.9% of the PNETs examined. However, all cases of SPN and PDAC were found to be negative, with a statistically significant correlation (P=0.000). Cao *et al.* (2023) demonstrated that 71.4% of PNETs were positive for CgA, while only 7% of SPNs were positive, and all cases of PDAC were negative.

The present study's results are consistent with those of Tiemann *et al.* (2006), who found that no SPN tested positive for chromogranin. The current study's 100% sensitivity and 76.9% specificity for CgA in PNETs are consistent with Cao *et al.* (2023) findings, which displayed 71.43% sensitivity and 92.8% specificity for CgA.

In this study, the combined expression of both SOX11 and chromogranin was found to be more sensitive and specific in distinguishing SPN from PNET (100% specificity and 90.9% sensitivity). This is consistent with Harrison *et al.* (2018), who stated that the combination of SOX11 positive/CG negative was the most sensitive

in the diagnosis of SPN (90.9% sensitivity and 100% specificity).

Image analysis and laboratory measurements of CA19-9 in the serum are currently the standard methods for early identification and prognosis prediction in PDAC. Regretfully, there are downsides and limits to all of these approaches, and there is currently no helpful screening test available (Ben *et al.*, 2010). This highlights the necessity of finding novel tumor-specific molecular markers that could aid in early detection or serve as a tumor target therapy.

Regarding L1CAM expression in the studied PDAC cases. It was higher expressed in 70% of pancreatic cancer than adjacent nontumors pancreatic tissue (P<0.05). Also, there was a statistically significant positive correlation between L1CAM expression and higher grade (P=0.02), advanced T stage (P=0.02), positive lymph node metastasis (P=0.002), positive LVI (P=0.000), and distant metastasis (P=0.046).

This agrees with the study of Wen *et al.* (2021) which revealed that the protein expression of L1CAM was associated with tumor size, degree of tumor differentiation, TNM stage, and lymph node metastasis (all P<0.05).

Chen *et al.* (2011) demonstrated that PDAC cells exhibit the NC adhesion protein L1. L1CAM expression was found in 92.7% of initial PDACs, 80% of lymph node metastases, and 100% of liver metastases according to immunohistochemistry investigations conducted by Bergmann *et al.* (2010).

In the same line Tsutsumi *et al.* (2011) found that a positive expression of L1CAM was significantly correlated with the histological grade, lymph node involvement, and distant metastasis. However, in the same study, L1CAM was only expressed only in 21.4% of PDAC cases.

When comparing pancreatic cancer tissues to their neighboring normal tissues, Ben *et al.* (2010) discovered that L1CAM was overexpressed in cancerous tissues. Significant node involvement (P=0.007), vascular invasion (P=0.012), and perineural invasion (P=0.001) were linked to positive L1CAM expression.

However, in contrast to this, another study group reported L1CAM expression in only 2% of PDAC (Kaifi *et al.*, 2006). The discrepancy between the two studies might be due to various experimental protocols, such as different criteria for determining L1CAM positivity and selecting cases, as well as potential sampling errors in smaller biopsies.

However, numerous in-vitro studies suggest that L1CAM plays a significant role in the initiation and progression of PDAC, contributing to the chemoresistant and migratory properties of tumor cells (Sebens Müerköster *et al.*, 2007; Geismann *et al.*, 2009).

Previous research on L1CAM's function in carcinomas has shown that it increases cell mobility on extracellular matrix components, enhances Matrigel invasion, and promotes tumor growth in immunodeficient mice (Thelen *et al.*, 2002; Gavert *et al.*, 2005).

The extracellular domain of L1CAM can be released from the cell surface through proteolytic cleavage, which promotes the migration and survival of tumor cells by binding to integrin through autocrine/paracrine signaling (Mechtersheimer *et al.*, 2001). Notably, L1CAM expression has been shown to affect gene expression, including Erk-dependent genes (Silletti *et al.*, 2004), and can contribute to the development of resistance to chemotherapy (Sebens Müerköster *et al.*, 2007).

Our research findings support the hypothesis that L1CAM plays a crucial role in the invasive process of PDAC and its expression is indicative of a more malignant phenotype, as previously demonstrated by Tsutsumi *et al.* (2011).

Conclusion

SOX11 is a highly sensitive marker for distinguishing between SPN, PNETs, and PDAC making it a potential target for developing therapeutic interventions for malignant pancreatic tumors. The combined expression of L1CAM, SOX11, and chromogranin may provide a useful role in addressing the diagnostic challenges associated with these tumors.

L1CAM may play a significant role in the progression, invasion, and metastasis of pancreatic adenocarcinoma and may have prognostic significance. Therefore, targeted therapies that address chemoresistance may be effective in treating these tumors.

Recommendation

A large-scale study using different molecular tests – other than the immunohistochemistry – on the role of L1CAM in PDAC is recommended.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

Anderson RB, Turner KN, Nikonenko AG, Hemperly J, Schachner M, Young HM. The cell adhesion molecule l1 is required for chain migration of neural crest cells in the developing mouse gut. Gastroenterology 2006;130:1221–1232.

Beà S, Amador V (2017). Role of SOX11 and genetic events cooperating with cyclin D1 in mantle cell lymphoma. Curr Oncol Rep 19:1.

Ben QW, Wang JC, Liu J, Zhu Y, Yuan F, Yao WY, *et al*. (2010). Positive expression of L1-CAM is associated with perineural invasion and poor outcome in pancreatic ductal adenocarcinoma. Ann Surg Oncol 17:2213–2221.

Bergmann F, Wandschneider F, Sipos B, Moldenhauer G, Schniewind B, Welsch T, *et al*. (2010). Elevated L1CAM expression in precursor lesions and primary and metastastic tissues of pancreatic ductal adenocarcinoma. Oncol Rep 24:909–915.

Bernard P, Harley VR (2010). Acquisition of SOX transcription factor specificity through protein–protein interaction, modulation of Wnt signalling and post-translational modification. Int J Biochem Cell Biol 42:400–410.

Cai Y, Ran X, Xie S, Wang X, Peng B, Mai G, *et al*. (2014). Surgical management and long-term follow-up of solid pseudopapillary tumor of pancreas: a large series from a single institution. J Gastrointest Surg 18:935–940.

Cao Z, Cen HB, Zhao JH, Mei J, Qin LZ, Liao W, *et al*. (2023). Expression and significance of INSM1 and SOX11 in pancreatic neuroendocrine tumor and solid pseudopapillary neoplasm. Beijing da xue xue bao. J Peking Univ Health Sci 55:575–581.

Chen MM, Lee CY, Leland HA, Silletti S (2011). Modification of the L1-CAM carboxy-terminus in pancreatic adenocarcinoma cells. Tumor Biol 32:347–357.

Cho JH, Hong SP, Park JY, Jeon TJ, Kim HM, Bang S, *et al*. (2008). W1412 expression of Sox-11 and Sox-4 is involved in pathogenesis of solid pseudopapillary tumor in pancreas. Gastroenterology 4:A-699.

Dinarvand P, Wang WL, Roy-Chowdhuri S (2022). Utility of SOX11 for the diagnosis of solid pseudopapillary neoplasm of the pancreas on cytological preparations. Cytopathology 33:216–221.

Ducreux M, Cuhna AS, Caramella C, Hollebecque A, Burtin P, Goéré D, *et al*. (2015). Cancer of the pancreas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 26:v56–v68.

Foo WC, Harrison G, Zhang X (2017). Immunocytochemistry for SOX-11 and TFE3 as diagnostic markers for solid pseudopapillary neoplasms of the pancreas in FNA biopsies. Cancer Cytopathol 125:831–837.

Gavert N, Conacci-Sorrell M, Gast D, Schneider A, Altevogt P, Brabletz T, *et al*. (2005). L1, a novel target of β-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. J Cell Biol 168:633–642.

Gavert N, Ben-Shmuel A, Raveh S, Ben-Ze'ev A (2008). L1-CAM in cancerous tissues. Expert Opin Biol Ther 8:1749–1757.

Geismann C, Morscheck M, Koch D, Bergmann F, Ungefroren H, Arlt A, *et al*. (2009). Up-regulation of L1CAM in pancreatic duct cells is transforming growth factor β1 – and slug-dependent: role in malignant transformation of pancreatic cancer. Cancer Res 69:4517–4526.

Global Cancer Observatory (2020). Cancer today. International Agency for Research on Cancer. Available at https://gco.iarc.fr/today (Accessed October 28, 2020).

Guo M, Luo G, Jin K, Long J, Cheng H, Lu Y, *et al*. (2017). Somatic genetic variation in solid pseudopapillary tumor of the pancreas by whole exome sequencing. Int J Mol Sci 18:81.

Harrison G, Hemmerich A, Guy C, Perkinson K, Fleming D, McCall S, *et al*. (2018). Overexpression of SOX11 and TFE3 in solid-pseudopapillary neoplasms of the pancreas. Am J Clin Pathol 149:67–75.

Hewitt MJ, McPhail MJ, Possamai L, Dhar A, Vlavianos P, Monahan KJ (2012). EUS-guided FNA for diagnosis of solid pancreatic neoplasms: a meta-analysis. Gastrointest Endosc 75:319–331.

Hochwald SN, Zee S, Conlon KC, Colleoni R, Louie O, Brennan MF, *et al*. (2002). Prognostic factors in pancreatic endocrine neoplasms: an analysis of 136 cases with a proposal for low-grade and intermediate-grade groups. J Clin Oncol 20:2633–2642.

Hua T, Liu S, Xin X, Jin Z, Liu Q, Chi S, *et al*. (2016). Prognostic significance of L1 cell adhesion molecule in cancer patients: a systematic review and meta-analysis. Oncotarget 7:85196.

Huszar M, Moldenhauer G, Gschwend V, Ben-Arie A, Altevogt P, Fogel M (2006). Expression profile analysis in multiple human tumors identifies L1 (CD171) as a molecular marker for differential diagnosis and targeted therapy. Hum Pathol 37:1000–1008.

Inaguma S, Wang Z, Lasota JP, Miettinen MM. Expression of neural cell adhesion molecule L1 (CD171) in neuroectodermal and other tumors. An immunohistochemical study of 5155 tumors and critical evaluation of CD171 prognostic value in gastrointestinal stromal tumors. Oncotarget 2016;7:55276.

Julian LM, McDonald AC, Stanford WL (2017). Direct reprogramming with SOX factors: masters of cell fate. Curr Opin Genet Dev 46:24–36.

Kaifi JT, Zinnkann U, Yekebas EF, Schurr PG, Reichelt U, Wachowiak R, *et al*. (2006). L1 is a potential marker for poorly-differentiated pancreatic neuroendocrine carcinoma. World J Gastroenterol 12:94.

Kim EK, Jang M, Park M, Kim H (2017). LEF1, TFE3, and AR are putative diagnostic markers of solid pseudopapillary neoplasms. Oncotarget 8:93404.

Kloppel G, Hruban RH, Klimstra DS, Maitra A, Morohoshi T, Notohara K, *et al*. (2010). WHO classification of tumors of the digestive system. 327.

Li P, Hu Y, Yi J, Li J, Yang J, Wang J (2015). Identification of potential biomarkers to differentially diagnose solid pseudopapillary tumors and pancreatic malignancies via a gene regulatory network. J Transl Med 13:1–4.

Liszka Ł (2021). L1 cell adhesion molecule (L1CAM) and nerve growth factor receptor (NGFR, p75) expression patterns in solid pseudopapillary neoplasm of the pancreas. Pol J Pathol 72:211.

Mansour JC, Chen H (2004). Pancreatic endocrine tumors. J Surg Res 120:139–161.

Mechtersheimer S, Gutwein P, Agmon-Levin N, Stoeck A, Oleszewski M, Riedle S, *et al*. (2001). Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. J Cell Biol 155:661–674.

Moradi A, Ghasemi F, Anvari K, Hassanian SM, Simab SA, Ebrahimi S, *et al*. (2017). The cross-regulation between SOX15 and Wnt signaling pathway. J Cell Physiol 232:3221–3225.

Ohara Y, Oda T, Hashimoto S, Akashi Y, Miyamoto R, Enomoto T, *et al*. (2016). Pancreatic neuroendocrine tumor and solid-pseudopapillary neoplasm: key immunohistochemical profiles for differential diagnosis. World J Gastroenterol 22:8596.

Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM (2014). Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 74:2913–2921.

Rawnaq T, Quaas A, Zander H, Gros SJ, Reichelt U, Blessmann M, *et al*. (2012). L1 is highly expressed in tumors of the nervous system: a study of over 8000 human tissues. J Surg Res 173:314–319.

Sebens Müerköster S, Werbing V, Sipos B, Debus MA, Witt M, Grossmann M, *et al*. (2007). Drug-induced expression of the cellular adhesion molecule L1CAM confers anti-apoptotic protection and chemoresistance in pancreatic ductal adenocarcinoma cells. Oncogene 26:2759–2768.

Silletti S, Yebra M, Perez B, Cirulli V, McMahon M, Montgomery AM (2004). Extracellular signal-regulated kinase (ERK)-dependent gene expression contributes to L1 cell adhesion molecule-dependent motility and invasion. J Biol Chem 279:28880–28888.

Springer S, Wang Y, Dal Molin M, Masica DL, Jiao Y, Kinde I, *et al*. (2015). A combination of molecular markers and clinical features improve the classification of pancreatic cysts. Gastroenterology 149:1501–1510.

Thelen K, Kedar V, Panicker AK, Schmid RS, Midkiff BR, Maness PF (2002). The neural cell adhesion molecule L1 potentiates integrin-dependent cell migration to extracellular matrix proteins. J Neurosci 22:4918–4931.

Tiemann K, Kosmahl M, Ohlendorf J, Krams M, Klöppel G (2006). Solid pseudopapillary neoplasms of the pancreas are associated with FLI-1 expression, but not with EWS/FLI-1 translocation. Mod Pathol 19:1409–1413

Tsutsumi S, Morohashi S, Kudo Y, Akasaka H, Ogasawara H, Ono M, *et al*. (2011). L1 Cell adhesion molecule (L1CAM) expression at the cancer invasive front is a novel prognostic marker of pancreatic ductal adenocarcinoma. J Surg Oncol 103:669–673.

van der Maten M, Reijnen C, Pijnenborg JM, Zegers MM (2019). L1 cell adhesion molecule in cancer, a systematic review on domain-specific functions. Int J Mol Sci 20:4180.

Wang Y, Fu Y, Zheng Z, Wu HY, Zhou Q, Chen KL, *et al*. (2020). Expression of SOX-11 and TFE3 in solid-pseudopapillary tumor of pancreas and its clinical significance. Chin J Pathol 49:1036–1040.

Wen QI, Taiwen CH, Zheng H, Xiaodong ZH (2021). Expression and significance of L1 cell adhesion molecule and transforming growth factor-β1 in pancreatic cancer tissue. J Clin Hepatol 37:1404–1408.

Wislet S, Vandervelden G, Rogister B (2018). From neural crest development to cancer and vice versa: how p75NTR and (Pro) neurotrophins could act on cell migration and invasion?. Front Mol Neurosci 11:244.

Ye J, Ma M, Cheng D, Yuan F, Deng X, Zhan Q, *et al*. (2012). Solid-pseudopapillary tumor of the pancreas: clinical features, pathological characteristics, and origin. J Surg Oncol 106:728–735.

Zhao T, Yang H, Tian Y, Xie Q, Lu Y, Wang Y, *et al*. (2016). SOX7 is associated with the suppression of human glioma by HMG-box dependent regulation of Wnt/β-catenin signaling. Cancer Lett 375:100–107.

Amin MB, Edge S, Greene F, Byrd DR, Brookland RK, Washington MK, *et al*. AJCC Cancer Staging Manual. 8th edition. New York: Springer; 2017.