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RESEARCH ARTICLE

Expression of PTEN and mTOR in pancreatic neuroendocrine tumors

Xu Han · Yuan Ji · Jing Zhao · Xuefeng Xu · Wenhui Lou

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Abstract The purposes of this study were to clarify the expression patterns of phosphorylated mammalian target of rapamycin (p-mTOR), mTOR, and phosphatase and tensin homolog (PTEN) in primary pancreatic neuroendocrine tumors (pNETs) and their significance in predicting clinical behaviors and postoperative outcomes. The expressions of p-mTOR, mTOR, and PTEN were assessed in 20 normal pancreatic islets and in 90 resectable pNETs using immunohistochemistry. The associations of the biomarker expressions with clinicopathologic variables and survival duration were analyzed. The percentages of G1, G2, and G3 tumors were 54.4, 43.3, and 2.2 %, respectively. A strongly positive staining was observed for both mTOR and PTEN in normal pancreatic islets, whereas negative staining was observed for p-mTOR. In primary pNETs, the mTOR and p-mTOR positive rates were 70.8 % (63/89) and 44.4 % (40/90), respectively. p-mTOR expressions strongly correlate with mTOR expressions. No significant correlation between p-mTOR and clinicopathological features was found. The high expression rate of PTEN was 56.7 % (51/90), whereas the low expression rate was 43.4 % (39/90). PTEN loss (low expression) was significantly more frequent in patients with advanced WHO grades (p=0.004) and in patients with higher Ki-67 index (p=0.002). In our immunohistochemical classification system, the Ki-67 index was significantly higher in the PTEN low expression/p-mTOR-positive subgroup (2.7±2.5) than in the PTEN high expression/p-mTOR-negative subgroup (1.0±1.7, p=0.006). Patients in the PTEN low expression/p-mTOR-positive subgroup presented a significantly lower 5-year overall survival (OS) than those in the PTEN high expression/p-mTOR-negative subgroup (p=0.049; 5-year OS=79 vs. 100 %, HR=7.0). ENETS TNM staging and major vascular invasion were independently associated factors for predicting the overall survival rate of patients (p=0.019 and 0.011, respectively). In conclusion, positive p-mTOR expression and PTEN loss may have a synergic effect on tumorigenesis and proliferation; targeted therapy based on mTOR/PTEN signal pathway and its associated molecular mechanism may play a role in the treatment of pancreatic neuroendocrine tumors.

Keywords Neuroendocrine tumor · Pancreas · mTOR · PTEN

X. Han · X. Xu · W. Lou (✉)
Department of Pancreatic Surgery, Zhong Shan Hospital, Fudan University, 180 Fenglin Road,
Shanghai 200032, China
e-mail: louzsh@126.com

Y. Ji · J. Zhao
Department of Pathology, Zhong Shan Hospital,
Fudan University, Shanghai, China

Introduction

Pancreatic neuroendocrine tumors (pNETs) are uncommon neoplasms that may be derived from pluripotent stem cells within the neuroendocrine characteristics. They account for about 1–2 % of all pancreatic neoplasms [1, 2], with a low overall incidence of <1.5 cases per 100,000 person-years in population studies [3–8]. According to the Surveillance, Epidemiology and End Results program and the National Cancer Registry of Spain, the prevalence of pNETs has substantially increased over the past decades. Pancreatic endocrine tumors are generally more indolent than adenocarcinoma of the pancreas and have a better prognosis. They tend to be slow-growing, although aggressive variants exist and often cause hormone hypersecretion and other symptoms. In our current study, we investigated the role of two

important and interconnected cancer signaling pathways of pNETs: the mammalian target of rapamycin (mTOR) and phosphatase and tensin homolog (PTEN).

mTOR belongs to the highly conserved 3-kinase-related protein kinase and plays a key role in cellular growth survival, metabolism, and development [9–12]. mTOR is a key downstream controller of the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and can be activated by phosphorylation through Akt via the PI3K/Akt pathway at Ser^{2448} and by autophosphorylation at the Ser^{2481} site [9, 10, 13, 14]. Overexpression of extracellular signals such as IGF-I and VEGF receptors may stimulate PI3K-Akt-mTOR cascade [15, 16]. Phosphorylated mTOR (p-mTOR) sequentially activates the downstream molecules. mTOR comprises two structurally distinct complexes: mTOR complex 1 (mTORC1) and complex 2 (mTORC2). The best-characterized substrates for mTORC1 are eIF4E-binding protein and p70 S6 kinase, whereas for mTORC2 are protein kinase B (Akt) and protein kinase C [17]. The activated downstream signal molecules participate in tumor growth, proliferation, angiogenesis, and metastases [18, 19]. mTOR is involved in several biological behaviors [20] and is responsible for the G1 to S phase cell cycle progression under appropriate growth conditions [9, 14, 20]. On the other hand, the PI3K/Akt /mTOR pathway itself is frequently dysregulated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors [21, 22]. The deregulation of mTOR and p-mTOR is found in many tumors and is thought to be related with poor clinical outcomes. Among the negative regulators of mTOR involved in pNETs, PTEN is a crucial tumor suppressor of the PI3K/Akt/mTOR pathway with a GTPase activating function that is potentially fascinating on account of its implication for therapy. By dephosphorylating PtdIns-3, 4, 5-P3, PTEN acts in opposition to PI3K. PTEN is frequently mutated or lost in several sporadic or familiar cancer types [23], while in pNETs the frequency of loss is between 10 and 29 % [24–26]. It has been preclinically shown that deficiency of TSC2 or PTEN expression induces impaired PI3K/Akt/mTOR activation, indicating that mTOR overexpression with the loss of PTEN plays a key role in the development and progression of pNETs [27].

Thus, mTOR is currently under investigation as a potential target for anticancer therapy. In recent years, RAD001 (Everolimus), an mTOR inhibitor, has shown promising efficiency for well-differentiated and moderately differentiated pNETs in a recent multicenter, randomized, double-blind, placebo-controlled phase III trial [28]. However, the effect of mTOR inhibitors on poorly differentiated neuroendocrine tumors such as neuroendocrine carcinoma (NEC) or mixed adenoneuroendocrine carcinomas (MANEC) has not been identified. Moreover, the expression of activated mTOR and loss of PTEN have not been investigated

Table 1 Clinicopathological features of 90 pNET patients

Clinicopathological features	*N*	Percentage
Sex		
Male	44	48.9
Female	46	51.1
Location of primary tumor		
Head of pancreas	33	36.7
Neck/body/tail of pancreas	52	57.8
Diffusion/retroperitoneum	5	5.6
Primary tumor		
Single tumor	84	93.3
Multifocal tumors	6	6.7
Functioning tumor		
Yes	22	(24.4
No	68	75.6
Detection of regional lymph node metastases		
Yes	18	41.9
No	25	58.1
Extrapancreatic organ invasion at diagnosis		
Yes	12	13.3
No	78	86.7
Nerve invasion		
Yes	22	24.4
No	68	75.6
Major vascular invasion		
Yes	12	13.3
No	78	86.7
Necrosis		
Yes	28	31.1
No	62	68.9
WHO classification		
NET	88	97.8
NEC	1	1.1
MANEC	1	1.1
WHO grade		
G1	49	54.4
G2	39	43.3
G3	2	2.2
ENETS TNM stage		
I	21	23.3
IIA	26	28.9
IIIB	15	16.7
IIIA	7	7.8
IIIB	12	13.3
VI	9	10.0
Liver metastases		
Yes	16	17.8
No	74	82.2
Ki-67 index		
≤2 %	60	66.7
>2 %	30	33.3

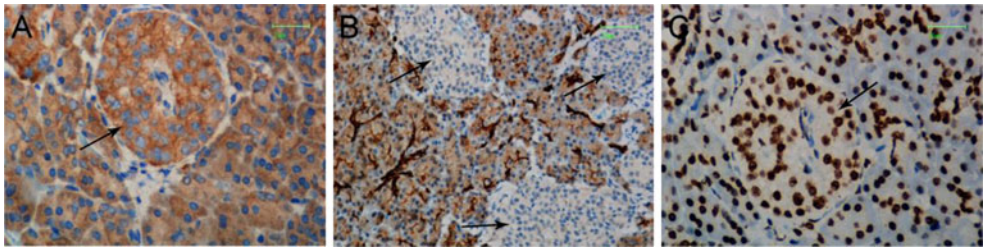

Fig. 1 Expressions of mTOR, p-mTOR, and PTEN in normal pancreatic islets (magnification, ×200–400). **a** mTOR was strongly positive in normal islet (*solid arrow*) and in normal acinar cells. **b** No expression of p-mTOR was found in normal islets (*solid arrows*), whereas p-mTOR was strongly positive in the exocrine cells of the pancreas. **c** PTEN was strongly positive in normal islet (*solid arrow*) and in normal acinar cells

extensively in pancreatic neuroendocrine tumors. It is still unclear whether the deficiency of PTEN with overexpression of p-mTOR synergistically promotes tumorigenesis process, cancer progression, and establishment of distant metastasis in pNETs. The purposes of our current study were to evaluate the expressions of mTOR and PTEN in pNETs and explore the relationship between mTOR/p-mTOR/PTEN expression and clinicopathological features and prognosis of pNETs.

Materials and methods

Patients and tissues

Samples from the resected specimen of pNETs were obtained between 1999 and 2011 from the Department of General Surgery, Zhongshan Hospital of Fudan University. Histological diagnosis was made according to the World Health Organization (WHO) classification [29]. In particular, the Ki-67 proliferative index is expressed as the percentage of Ki67-positive cells in 2,000 tumor cells within areas of the highest immunostaining using the MIB1 antibody (Dako, Glostrup, Denmark). Mitotic count is determined by counting 50 high-power fields (HPFs) and in the area of the highest mitotic activity and reported as the number of mitoses per 10 HPFs. All tumors were evaluated and classified initially by conventional hematoxylin and eosin (H&E) sections and their neuroendocrine nature was confirmed immunohistochemically using the neuroendocrine markers chromogranin A and synaptophysin. Additionally, tumors were selectively examined for their reactivities for gut hormone peptide antibodies including gastrin and insulin. Subsequently, based on the intraoperative, pathological, and radiological findings, patients were also classified according to the European Neuroendocrine Tumor Society (ENETS) tumor node metastasis (TNM) classifications [30]. Follow-up information was collected from clinical records, surgical and pathologic reports, radiology examination results, and the Tumor Registry at Zhongshan Hospital of Fudan University.

Regarding pNETs, patients with clinical symptoms and elevated plasma hormone levels were diagnosed as having functioning pNETs. On the other hand, patients without clinical symptoms and with no elevation of plasma hormone levels were diagnosed as having non-functioning pNETs, regardless of whether hormone production was evaluated by immunohistochemistry.

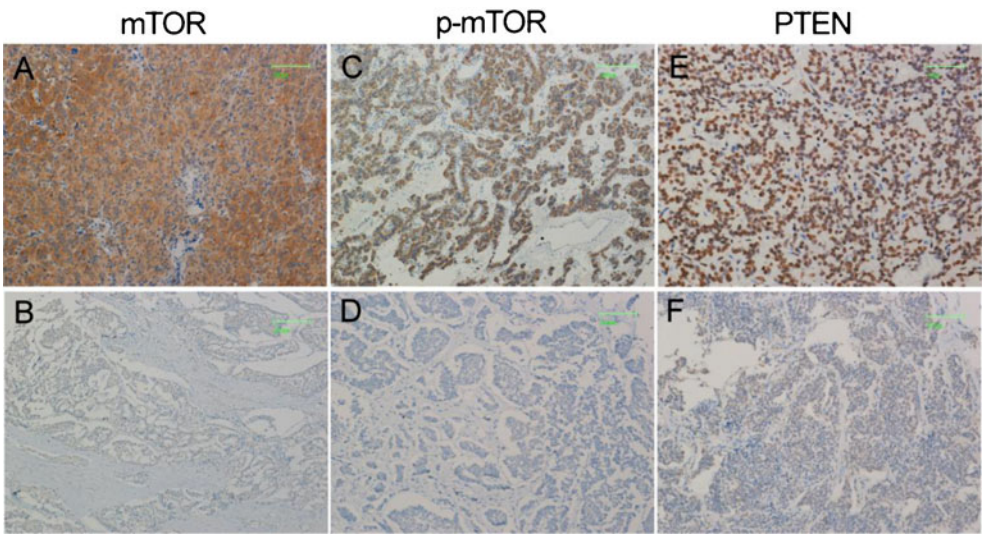

Fig. 2 Expression patterns of mTOR, p-mTOR, and PTEN (magnification, ×50–200). **a** An mTOR strongly positive sample. Cytoplasmic and/or membranous staining was observed. **b** An mTOR-negative sample. **c** A p-mTOR strongly positive sample. Cytoplasmic and/or membranous staining was observed. **d** A p-mTOR-negative sample. **e** Sample with high expression of PTEN. Nuclear and/or cytoplasmic strong staining was observed. **f** Sample with low expression of PTEN

Table 2 Immunohistochemical expressions of p-mTOR, mTOR, and PTEN

Immunohistochemical expression	Low expression, *N* (%)		High expression, *N* (%)	
	Negative	Weakly positive	Medium positive	Strongly positive
p-mTOR	50 (55.6)	25 (27.8)	10 (11.1)	5 (5.6)
mTOR	26 (29.2)	12 (13.5)	13 (14.6)	38 (42.7)
PTEN	2 (2.2)	37 (41.7)	48 (53.3)	3 (3.3)

Immunohistochemistry

Twenty samples with fully normal pancreatic islets excluding malignant neoplasms, pancreatic intraepithelial neoplasia, and chronic pancreatitis were identified as the normal control group. To ensure uniform staining conditions of the tumor among all samples, the tissue microarray method was elected. Sections (4 μm) were cut from formalin-fixed paraffin-embedded tissues and placed onto silanized slides. Slides were then stained using the Bond-Max Leica autostainer (Leica Biosystems, Milton Keynes, UK). Antibody detection was performed using the biotin-free Bond Polymer refined Detection System (DS9800, Leica Microsystems, Newcastle, UK) according to the manufacturer's protocol. Immunohistochemistry was performed in 90 cases for p-mTOR, 90 cases for PTEN, and 89 cases for mTOR (tumor lesion was not sectioned in one case). The antibodies used were as follows: p-mTOR (dilution, 1:200; Cell Signaling Technology, USA), mTOR (dilution, 1:200; Cell Signaling Technology), and PTEN (dilution, 1:200; Santa Cruz Biotechnology, USA). For p-mTOR and mTOR, positive staining was considered when cytoplasmic and/or membranous staining was observed. Immunoreactivity was evaluated on a semiquantitative scale considering both the extent (score, 0–3 for positive cells, <5, 5–40, 40–70, and >70 %, respectively) and the intensity (score, 0–3 for "–", "+", "++", and "+++", respectively) of staining. The product was used to obtain a total immunostaining score (range, 0–12). Samples with a score of 0 were considered as "negative," 1 or 2 as "weakly positive," 3 or 4 as medium positive, and 6–9 considered strongly positive. For PTEN, positive staining was scored when nuclear and/or cytoplasmic staining was observed. As described above, immunoreactivity was also evaluated on a semiquantitative scale. Here, samples with a score of 0 were considered as negative, 1–4 as weakly positive, 6 considered medium positive, and a score 9 considered strongly positive. For all biomarkers, samples with negative or weakly positive staining were considered to have low expression; others with medium positive or strongly positive staining were considered to have high expression.

Table 3 Correlation of p-mTOR expression with clinicopathological features

Clinicopathological features	*n*	Negative	Positive	*p* value
Function				0.062
Functioning tumors	22	16	6	
Non-functioning tumors	68	34	34	
Detection of regional lymph node metastases				0.268
Yes	18	11	7	
No	25	11	14	
ENETS TNM stage				0.476
I–II	62	36	26	
III–IV	28	14	14	
WHO grade				0.750
G1	49	29	20	
G2	39	20	19	
G3	2	1	1	
Liver metastases				0.279
Yes	16	11	5	
No	74	39	35	
Ki-67 index				0.230
≤2 %	60	36	24	
>2 %	30	14	16	
Ki-67 index (median±SD)		1±2.6	0±3.4	0.605

Table 4 Correlation of PTEN expression with clinicopathological features

Clinicopathological features	*n*	Low expression	High expression	*p* value
Function				0.468
Functioning tumors	22	11	11	
Non-functioning tumors	68	28	40	
Detection of regional lymph nodes metastases				0.268
Yes	18	9	9	
No	25	11	14	
ENETS TNM stage				0.690
I–II	62	26	36	
III–IV	28	13	15	
WHO grade				0.004
G1	49	14	35	
G2	39	23	16	
G3	2	2	0	
Liver metastases				0.970
Yes	16	7	9	
No	74	32	42	
Ki-67 index				0.002
≤2 %	60	19	41	
>2 %	30	20	10	
Ki-67 index (median±SD)		3±3.9	0±2.1	0.016

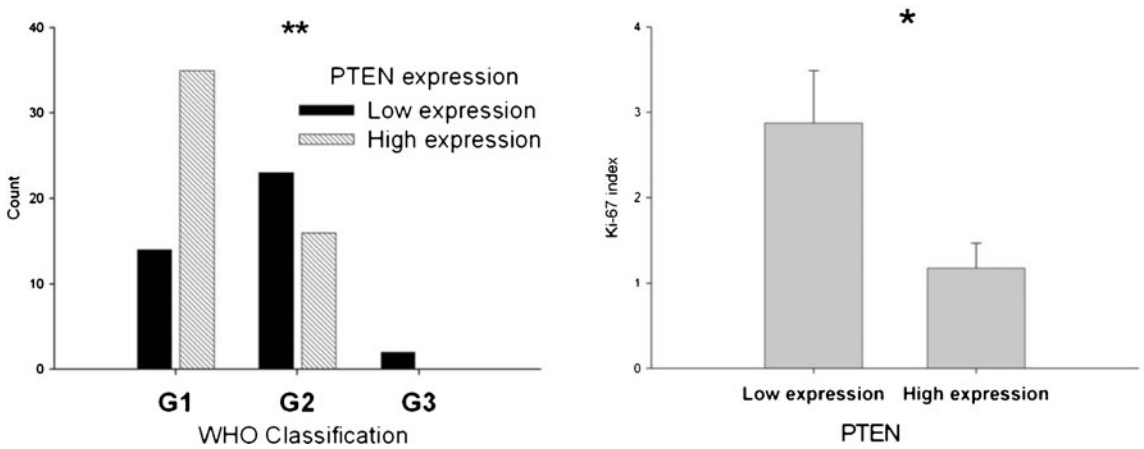

Fig. 3 *Left* PTEN loss was significantly more frequent in advanced WHO grade. *Right* Ki-67 index correlates with PTEN expression (*$p<0.05$; **$p<0.01$)

Statistical analysis

All analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Student's *t* test, Pearson's chi-square (χ^2) test, Fisher's exact test, Pearson's correlation test, and Spearman's correlation test were used to evaluate the association among variables when appropriate. The duration of overall survival (OS) was calculated from diagnosis until death or until the date of the last follow-up visit for patients still alive. Survival was estimated according to the Kaplan–Meier product limit method and Life Tables method. Survival curves were compared using the log-rank test. Possible prognostic factors associated with survival probability were considered in a multivariable Cox's proportional hazard regression analysis. Results are considered significant when $p<0.05$ was obtained.

Results

Clinicopathological features

Clinicopathological features of the 90 patients are listed in Table 1. The percentages of G1, G2, and G3 tumors were 54.4, 43.3, and 2.2 %, respectively. Neuroendocrine tumors were detected in 88 patients (97.8 %). The percentages of TNM stage I, stage II, stage III, and stage IV tumors were 23.3, 45.6, 21.1, and 10.0 %, respectively. The median tumor size was 3.6 cm (range, 0.8–18.0 cm). The median age at onset was 52 years (range, 9–79 years). Of 16 cases with liver metastases, nine had synchronous liver metastases and seven had metachronous liver metastases. Twenty-two pNETs (24.4 %) were functioning, whereas 68 pNETs (75.6 %) were nonfunctioning. All 90 patients received radical resection. The overall 1-, 2-, and 5-year accumulative survival rates were 98 % (95% CI=97–99 %), 95 % (95% CI=92–98 %), and 92 % (95% CI=87–97 %), respectively. The median survival time was 139.8 months (95% CI=117.5–162.1 months).

Immunohistochemical results

In the normal control group, mTOR was strongly positive in normal islet cells and in normal acinar cells. In contrast, no p-mTOR staining was detected in normal islet cells, but p-mTOR was strongly positive in normal acinar cells and ductal epithelial cells. Meanwhile, PTEN was strongly positive in normal islet cells and in normal exocrine cells (Fig. 1). As mentioned above, tumors with more than 5 % positive cells are considered as positive. For both mTOR and p-mTOR, positive staining was considered when cytoplasmic and/or membranous staining was observed; for PTEN, positive staining was considered when nuclear and/or cytoplasmic staining was observed (Fig. 2).

Among all pNET cases, the positive rates for p-mTOR, mTOR, and PTEN were 44.4 % (40/90), 70.8 % (63/89), and 97.8 % (88/90), respectively. On the other hand, the expression rates of p-mTOR, mTOR, and PTEN were 16.7 % (15/90), 57.3 % (51/89), and 56.7 % (51/90), respectively (Table 2). In addition, Spearman's rank correlation analysis indicated that p-mTOR expression strongly correlates with mTOR expression ($R=0.320$, $p=0.002$). However, a significant correlation between the p-mTOR and PTEN levels was not observed ($p>0.05$).

Relationships between the expressions of biomarkers and the clinicopathological features

In our study, no significant correlation between p-mTOR and clinicopathological features including function status, TNM stage, WHO grade, regional lymph node metastases,

Table 5 Univariate analysis of factors associated with overall survival in patients with pNETs

Parameter	Number of cases	Overall survival	
		5-year OS rate (%)	*p* value
Function status			
Functioning tumors	22	100	0.346
Non-functioning tumors	68	90	
Detection of regional lymph node metastases			
Yes	18	63	0.040
No	25	96	
ENETS TNM stage			
I–II	62	98	0.009
III–IV	28	77	
Liver metastases			
Yes	16	91	0.423
No	74	92	
Tumor diameter (cm)			
<2	20	100	0.204
≥2	70	90	
Ki-67 index			
≤2 %	60	93	0.511
>2 %	30	91	
Major vascular invasion			
Yes	12	67	0.004
No	78	97	
Mitotic count			
<2	50	100	0.001
≥2	30	74	
Necrosis			
Yes	28	81	0.048
No	62	96	
Age			
>60 years	23	81	0.219
≤60 years	67	97	
mTOR			
Negative	26	84	0.038
Positive	63	96	
p-mTOR			
Negative	50	96	0.414
Positive	40	86	
PTEN			
High expression	51	98	0.077
Low expression	39	83	

liver metastases, and Ki-67 index was noted (Table 3). Also, there was no significant correlation between p-mTOR and clinicopathological features including function status, TNM stage, WHO grade, regional lymph node metastases, and liver metastases (Table 4). Interestingly, patients with advanced grades had a significantly lower PTEN expression (Pearson's chi-square test: $p=0.004$; Spearman's correlation test: $R=-0.339$, $p=0.01$). In addition, PTEN loss in patients with high Ki-67 index as a categorical variable and a continuous variable was more frequent than that in patients with low Ki-67 index ($p<0.05$; Fig. 3).

Correlation between the expressions of biomarkers and the prognosis

Univariate analysis showed that patient's regional lymph node metastases, ENETS TNM staging, major vascular invasion, mitotic count, necrosis, and mTOR expression correlated with the prognosis ($p=0.040$, 0.009, 0.004, 0.001, 0.048, and 0.038, respectively; Table 5). The results showed that ENETS TNM staging and major vascular invasion were independently associated factors for predicting the overall survival rate of patients ($p=0.019$ and 0.011, respectively; Table 6).

Discussion

In sporadic cases of pancreatic neuroendocrine tumors, abnormalities in the mTOR pathway have been identified [31]. Loss of PTEN, a negative regulator of the PI3K–Akt–mTOR pathway, has been associated with human disease, including cancer. The role of PTEN in tumorigenesis, cancer progression, and response to cancer therapies has been widely explored. Loss of PTEN protein expression is found to be correlated with poor prognosis in various tumors. The deficiency of PTEN protein has been associated with resistance to the EGFR inhibitors gefitinib and erlotinib [32, 33]. Abnormalities in PTEN have also been observed in patients with sporadic pancreatic neuroendocrine tumors [27]. Evidence also suggests that the genetic abnormalities in the mTOR pathway may be critical in the development of some neuroendocrine tumors. Patients with genetic mutations and loss in PTEN, TSC2, NF1, and vHL genes have implicated the role of the PI3K–Akt–mTOR pathway in neuroendocrine tumors, the hereditary loss of these genes being associated with the development of neuroendocrine tumors. Neuroendocrine tumors are known to co-express both IGF-I and IGR, and exogenous IGF may activate mTOR and increase cellular proliferation in carcinoid cell lines [9, 16]. PTEN are inhibitors of mTOR that are normally expressed in neuroendocrine cells [31]. The function of mTOR and PTEN in the PI3K/AKT pathway makes them both potential biomarkers for response to several new molecular-targeted therapeutics. It has been proposed that deficiency of PTEN with overexpression of p-mTOR might synergistically predict poor prognosis [34–36]; however, it has not been verified in studies with larger sample sizes.

Immunohistochemistry has been proven to be a useful way for measuring p-mTOR and PTEN status. The

Table 6 Multivariate analysis of OS in patients with pNETs

Factors	Characteristics		Hazard ratio	95% CI	*p* value
	Unfavorable	Favorable			
Function status	Non-functioning	Functioning	2.625	0.326–21.113	0.364
Detection of regional lymph node metastases	+	−	4.803	0.921–25.033	0.062
ENETS TNM stage	III–IV	I–II	5.333	1.324–21.488	0.019
Liver metastases	+	−	1.905	0.383–9.488	0.431
Tumor diameter (cm)	≥2	<2	2.478	0.954–21.336	0.416
Ki-67 index	>2 %	≤2 %	1.788	0.309–10.347	0.516
Major vascular invasion	+	−	5.773	1.501–22.196	0.011
Mitotic count	≥2	<2	7.262	0.133–26.079	0.149
Necrosis	+	−	3.819	0.927–15.772	0.063
Age	>60 years	≤60 years	2.240	0.598–8.396	0.232
mTOR	Negative	Positive	3.947	0.979–15.910	0.054
p-mTOR	Positive	Negative	2.789	0.435–7.354	0.420
PTEN	Low expression	High expression	3.268	0.815–13.111	0.095

immunochemical results in our current study showed that the positive rates for p-mTOR and mTOR were 44.4 % (40/90) and 70.8 % (63/89). Similar positive rates for p-mTOR and mTOR in pNETs were found in other retrospective studies [27, 34, 37]. The results of this study demonstrate that mTOR was expressed in a high percentage (70.8 %), but mTOR was expressed in a lower percentage (44.4 %). These results might reflect an obvious preponderance of G1/G2; however, the distribution of patients with pancreatic neuroendocrine carcinomas expressing mTOR is still unknown. PTEN was positive in 97.8 % (88/90) of tumors, whereas the PTEN level was downregulated in 39 cases (43.4 %) in our center. Staining of PTEN was altered in either nuclear, cytoplasmic, or both cell compartments. In our future study, pNETs with G3 or advanced stage merit further identification. The expression of PTEN was correlated with the WHO grade, and patients classified as advanced grades had lower expressions of PTEN (p=0.01). These results were consistent with those reported in a recent gene expression profiling study [27]. In studies with larger sample sizes, PTEN and TSC2 were downregulated in most primary tumors examined. Downregulation was significantly associated with shorter disease-free and overall survival, supporting a role for the PI3K/Akt/mTOR pathway in the development of pNETs (Fig. 4).

In our current study, although no statistically significant difference was found, a high expression of PTEN seemed to prolong survival duration. Similarly, patients with positive

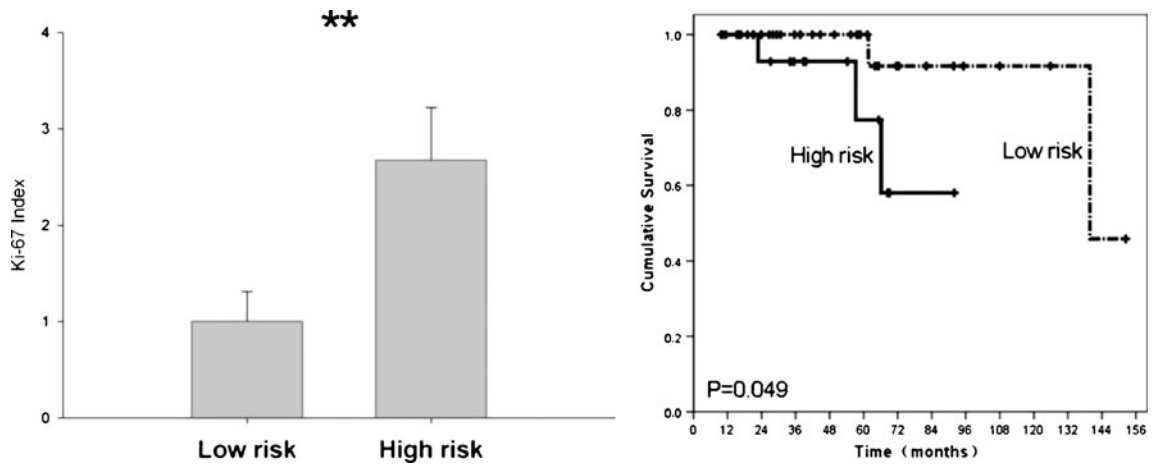

Fig. 4 *Left* Ki-67 index correlates with risk level. *Right* Overall survival between "high-risk" and "low-risk" patients (5-year OS=79 vs. 100 %)

p-mTOR expression seemed to have shorter survival duration than those with negative p-mTOR expression. On the basis of these findings, a three-tiered immunohistochemical classification system derived from the expression of p-mTOR and PTEN was developed, and it correlated with survival and tumor proliferation. The extended survival analysis showed the predictive value of p-mTOR with PTEN: survival duration was significantly greater in the "low-risk" (PTEN high expression/p-mTOR-negative) than the "high-risk" (PTEN low expression/positive p-mTOR expression) subgroup. It is therefore concluded that positive p-mTOR expression and PTEN loss may have synergic effects on the tumorigenesis process by activating the mTOR signal pathway related to poor prognosis. Meanwhile, the Ki-67 index was significantly higher in the high-risk subgroup than in the low-risk subgroup, suggesting that positive p-mTOR expression and PTEN loss correlated with increased cell growth and proliferation index. Our data on p-mTOR and PTEN supported the hypothesis of the involvement of the PI3K/Akt/mTOR signal pathway in pNET tumorigenesis and proliferation. With effective prognostic predictors of postoperative outcomes, the predictive value of p-mTOR combined with PTEN should be further investigated, especially in patients with pancreatic neuroendocrine carcinomas and mixed adenoendocrine carcinoma.

The expressions of the biomarkers in poorly differentiated endocrine carcinomas merit further investigations. In a recent RADIANT-3 trail, the largest ever placebo-controlled phase III clinical trial on pNETs that enrolled 410 patients with advanced pNETs, the mTOR inhibitor Everolimus provided a statistically and clinically significant 2.4-fold improvement in median progression-free survival (PFS) [28]. In the RADIANT-2 trail, which enrolled 429 patients with advanced NETs, Everolimus plus octreotide LAR provided a clinically meaningful 5.1-month improvement in median PFS compared to placebo plus octreotide LAR [38]. To date, two clinical trials have confirmed that pNETs had been linked to genetic alterations that activate the mTOR pathway. Octreotide downregulates IGF-1, an upstream activator of the PI3K/AKT/mTOR pathway, providing a durable benefit and acceptable safety profile [39]. We also found that ENETS TNM classification and the status of major vascular invasion can differentiate the prognosis significantly in the current study, which was consistent with previous studies [30, 40].

We are well aware that this is a retrospective study and, of course, our results do not have the same strength as an observational study; however, it provides a substantial basis for the design of future randomized, prospective clinical trials and treatment strategies.

In summary, positive p-mTOR expression and PTEN loss may have synergic effects on tumorigenesis and proliferation; targeted therapy based on the mTOR/PTEN signal pathway and its associated molecular mechanism may play a role in the treatment of pancreatic neuroendocrine tumors.

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Conflicts of interest The authors indicated no potential conflicts of interest.

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