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Prognostic Significance of CD44 and Orthopedia Homeobox Protein (OTP) Expression in Pulmonary Carcinoid Tumours

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Abstract CD44 and orthopedia homeobox protein (OTP) expressions have shown to be predictive of overall survival in pulmonary carcinoid (PC) tumours. The scope of the present study was to validate their role in PC patients and investigate potential application in clinical practice. Data was collected from patients presenting to a tertiary cancer centre diagnosed with PC between 2003 and 2015. Diagnosis was confirmed by central pathology review. Formalin-fixed paraffin-embedded (FFPE) tissue samples collected at diagnosis were scored using immunohistochemistry (*H* score) for standard CD44 and nuclear and cytoplasmic OTP protein expression. The study included 108 patients. High CD44/nuclear OTP (nOTP) expression was strongly associated with typical carcinoid (TC) histology ($p < 0.001$). Eighty-six patients, who underwent radical surgical resection, were selected to assess the impact of patient and tumour parameters on relapse-free survival (RFS). Sixty-nine (80 %) had TC and 17 (20 %) had atypical carcinoid tumours. On multivariate analysis, high CD44 and nOTP expression, TC histology and non-infiltrative tumour growth were associated with superior RFS. Early stage TC (stage pT1aN0) patients ($N = 32$; 46 %) had excellent prognosis irrespective of CD44/nOTP status. Importantly, TC patients with locally advanced disease (defined as >pT1aN0) and high CD44/nOTP expression ($N = 26$; 38 %) had excellent RFS ($p = 0.005$) compared to those with the same stage but low CD44 and/or nOTP ($N = 11$; 16 %). Additionally, the combination of CD44/nOTP expression and tumour growth pattern led to a more accurate prognostic system compared to the established WHO classification of PC tumours (concordance index = 0.902 vs 0.811, respectively, $p < 0.001$). Assessment of CD44/nOTP expression combined with tumour growth pattern identifies clear groups with largely different prognosis. These findings provide important information on how patients with these resected cancers should be followed up.

Keywords Pulmonary carcinoid tumours · Neuroendocrine · Prognosis · CD44 · Orthopedia homeobox protein

Introduction

Pulmonary carcinoid (PC) tumours represent 1–2 % of all lung primary cancers [1] with an age-adjusted incidence rate of 0.2–2.0 per 100,000 population per year [2]. Historically, PCs were considered to represent indolent neoplasms; however, they are now recognised to have locally invasive and metastatic potential [1, 2]. PCs represent low to intermediate neuroendocrine tumours of the lung, as opposed to high-grade neuroendocrine carcinomas such as small cell (SCLC) and large cell neuroendocrine carcinomas (LCNEC). However, their genetic profile and their disease course largely differ

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from that of neuroendocrine carcinomas, meaning that they should be managed as a separate entity [3, 4].

A landmark in the clinical management of these tumours was their classification into typical (TC) and atypical (AC) carcinoids (World Health Organisation (WHO) Classification of Tumours of the Lung, Pleura, Thymus and Heart [5]). PCs which demonstrate a mitotic index of 2–10 mitoses per 2 mm^2 and/or necrosis are defined as AC. AC commonly present at advanced stage, including lymph node infiltration and distant metastasis, relapse more often after surgical resection and progress more quickly when metastatic [1, 6]. By contrast, TC, defined by less than 2 mitoses per 2 mm^2 and absence of necrosis, have a more indolent course. This classification has a practical impact on the management of these tumours, with AC requiring more frequent follow-up and more aggressive treatment and TC being more often treated with active surveillance or non-toxic treatments [2].

Despite this widely used stratification, the current histopathology classification and tumour staging system do not exactly correlate with the biology and natural history of PCs [2, 4]. A number of potential prognostic factors and biomarkers have been proposed, but none of these have been validated for clinical use to date [3]. Recently, CD44 and orthopedia homeobox protein (OTP) have been demonstrated as promising biomarkers for PCs [7, 8]. CD44 is an adhesion molecule, which mediates cancer cellular adhesion, invasion and metastasis across a number of tumour types [9]. OTP represents a transcription factor implicated in the development of the hypothalamus during embryogenesis [10]. However, its role in adult life is unclear. CD44 and OTP protein expressions have been shown to predict overall survival in mixed groups of lung carcinoid patients, including early stage and metastatic cases [7, 8].

The scope of the present study was to validate the role of CD44 and OTP in PC patients and describe their potential application in the current clinical practice.

Patients and Methods

Patients

In this study, institutional, tumour board and pathology databases were searched for identification of patients diagnosed with pulmonary carcinoid (PC) tumours between February 2003 and November 2015. In total, 108 were eligible; the selection process is described in the CONSORT diagram in Fig. S1. Briefly, for inclusion, patients had central pathology confirmation of the diagnosis of pulmonary carcinoid tumour, available baseline and follow-up clinical data and adequate tumour tissue for further biomarker investigation. All patients were operated by a single surgeon (P.K.) in the Department of Cardiothoracic Surgery, University Hospital of South Manchester and were followed up postoperatively in the Department of Medical Oncology, Christie NHS Foundation Trust. A pathologist (D.N.) performed the pathology review and biomarker assessment of all tumour samples and was blinded for the patient outcome data (see below "Histopathology Evaluation" section).

All patients had baseline serum chromogranin A and 5-hydroxyindoleacetic acid (5-HIAA), whole-body octreotide or gallium scan and computed tomography (CT) scan of the chest, abdomen and pelvis. Patients were followed up clinically and radiologically by CT scan according to their pathology stage and histology.

All patients included in the study were used for correlative biomarker analysis. To avoid potential bias, only those with complete cancer resection (R0 or R1 resection) at surgery were included in analysis of prognostic factors.

All procedures have been conducted in accordance with the Helsinki Declaration, as revised in 2013.

Histopathology Evaluation

All histopathological specimens underwent central pathology review to confirm diagnosis according to the recent WHO classification [5].

Immunohistochemistry was performed on deparaffinised, formalin-fixed 4-μm tissue sections using rabbit anti-OTP polyclonal antibody (HPA039365, 1:150, Atlas Antibodies, Stockholm, Sweden). Antibody incubation and detection for OTP were carried out at room temperature on a Leica BOND staining platform (pre-treatment BOND ER 1, 30 min high temperature) with detection using Leica BOND Refine Staining kit (Leica Biosystems). Immunohistochemistry staining for CD44s (clone DF1485, 1:20, Leica), TTF1 (clone 8G7G3/1, 1:200, Dako) and Ki67 (clone MIB-1, 1:50 Dako) was performed as previously described [8, 11]. Appropriate positive and negative controls were included with the study sections (Fig. S2).

Membranous staining for CD44 and cytoplasmic and nuclear staining for OTP (cOTP and nuclear OTP (nOTP), respectively) were scored using *H* score method. Briefly, *H* score is obtained by the formula 3 × % of strongly staining cells + 2 × % of moderately staining cells + 1 × % of weakly staining cells, giving a range of 0 to 300. Any nuclear positive staining was regarded as being positive for TTF1.

The assessment of mitotic count and Ki-67 index was based on the European Neuroendocrine Tumour Society (ENETS) scheme for gastrointestinal neuroendocrine tumours [12]. Briefly, the mitotic count in 2 mm^2 that is 10 high power fields (×400) by NIKON Eclipse was evaluated in areas of highest mitotic density. Ki-67 indices were calculated as a percentage of Ki-67-positive cells in 500–2000 cells that were counted in areas of strongest nuclear labelling ("hot spots").

Statistical Analysis

The statistical analysis was performed using Statistical Package for the Social Sciences for Windows version 20 (SPSS, Inc., Chicago, IL). Parametric and non-parametric tests were used in the analysis. Receiver operating characteristic (ROC) curve was used to estimate the diagnostic accuracy of biomarkers. Median relapse-free survival (RFS) was calculated by Kaplan-Meier curves. RFS was defined as the time from surgery to disease relapse or death from other causes, whichever occurred first. Univariate analysis of prognostic significance was performed by log-rank tests for categorical variables and Cox-regression for continuous variables. The clinicopathological variables tested were age (<60 vs ≥60 years), gender, smoking status (non-smoker vs ex-smoker vs current smoker), ECOG performance status, histological subtype (typical vs atypical), Ki-67 index (<5 % vs ≥5 %), tumour growth type (infiltrative vs non-infiltrative), presence of diffuse pulmonary neuroendocrine hyperplasia (yes vs no), TTF-1 expression (positive vs negative), CD44/OTP expression (above or below median), primary tumour location (central vs peripheral), pathological stage (pT1N0 vs >pT1N0) and surgical resection margin (R0 vs R1). Multivariable Cox-regression model analysed baseline variables for possible prognostic significance, with a forward selection procedure and a removal criterion of $p > 0.10$. The concordance index (c index) was calculated to assess the performance of the prognostic models. All analyses were two-tailed and p values of ≤0.05 were considered significant.

Table 1 Basic patient and disease characteristics (entire population)

Characteristics	*N* (%)
Age (years)	
Median (range)	62 (21–83)
Gender	
Male	44 (41)
Female	64 (59)
PS (ECOG)	
0	71 (65)
1	31 (29)
2	4 (4)
3	2 (2)
Smoking	
No	52 (48)
Ex	35 (32)
Current	21 (20)
Stage	
Non-metastatic	92 (85)
Metastatic	16 (15)
Mitotic index	
Median (range)	0.5 (0.5–9.0)
Necrosis	
Yes	8 (7)
No	99 (92)
NA	1 (1)
Ki-67 (%)	
Median (range)	1.8 (0–15.0)
DIPNECH	
Yes	8 (7)
No	100 (93)
TTF-1	
Positive	54 (50)
Negative	54 (50)
Total	108 (100)

DIPNECH diffuse pulmonary neuroendocrine hyperplasia, *PS(ECOG)* performance status according to the Eastern Cooperative Oncology Group scale

Results

Basic Characteristics

Basic patient characteristics are shown in Table 1. The majority of patients were females, non-smokers, had excellent PS and non-metastatic disease stage. Pathology results of 86 patients who underwent radical tumour surgical resection are demonstrated in Table 2. Most of the tumours were TC and stage T1, while the majority of resections were on tumour-free

Table 2 Tumour characteristics in patients who underwent radical operation

Characteristics	*N* (%)
Histology	
Typical	69 (80)
Atypical	17 (20)
T stage	
T1	47 (55)
T2	33 (38)
T3	4 (5)
T4	2 (2)
Regional LN	
N0	65 (75)
N1	16 (19)
N2	5 (6)
Surgical margins	
R0	71 (83)
R1	15 (17)
Primary tumour location	
Central	53 (62)
Peripheral	33 (38)
Total	86 (100)

surgical margins (R0). All 86 patients had no evidence of disease on postoperative imaging tests.

Expression of CD44 and OTP

The distribution of CD44, nOTP and cOTP IHC expression levels (*H* score) is demonstrated in Fig. 1. The expression of CD44 was biphasic. Median *H* score was 145, range 0–300, 25 cases (23 %) had *H* score = 0 and 22 cases (20 %) had *H* score = 300. Median nOTP score was 300 (range, 0–300), 20 cases (18 %) had *H* score = 0 and 64 cases had *H* score = 300 (59 %). cOTP expression was low. Median score is 0 (range, 0–300) and 70 cases (65 %) had *H* score = 0, while only 1 (1 %) had *H* score = 300 and 8 (7 %) had *H* score = 210–300.

CD44 was positively correlated with nOTP expression (Spearman's rho = 0.517, $p < 0.001$), whereas nOTP was negatively correlated with cOTP (Spearman's rho = 0.429, $p < 0.001$). CD44 was not correlated with cOTP.

Correlation of CD44 and OTP Expression with Basic Patient and Tumour Characteristics

Correlations of CD44, nOTP and cOTP expression (*H* score) with basic patient and tumour characteristics are demonstrated in Table S1. High CD44 *H* score was associated with non-smoking history while high nOTP and low cOTP with female gender. With regard to histopathological characteristics, high CD44 and nOTP expressions were associated with typical histology (Fig. S3). Also, high nOTP *H* scores were positively correlated with TTF-1 expression. Centrally located primary tumours showed higher nOTP expression than peripherally located primaries (Table S2). Importantly, CD44, nOTP or

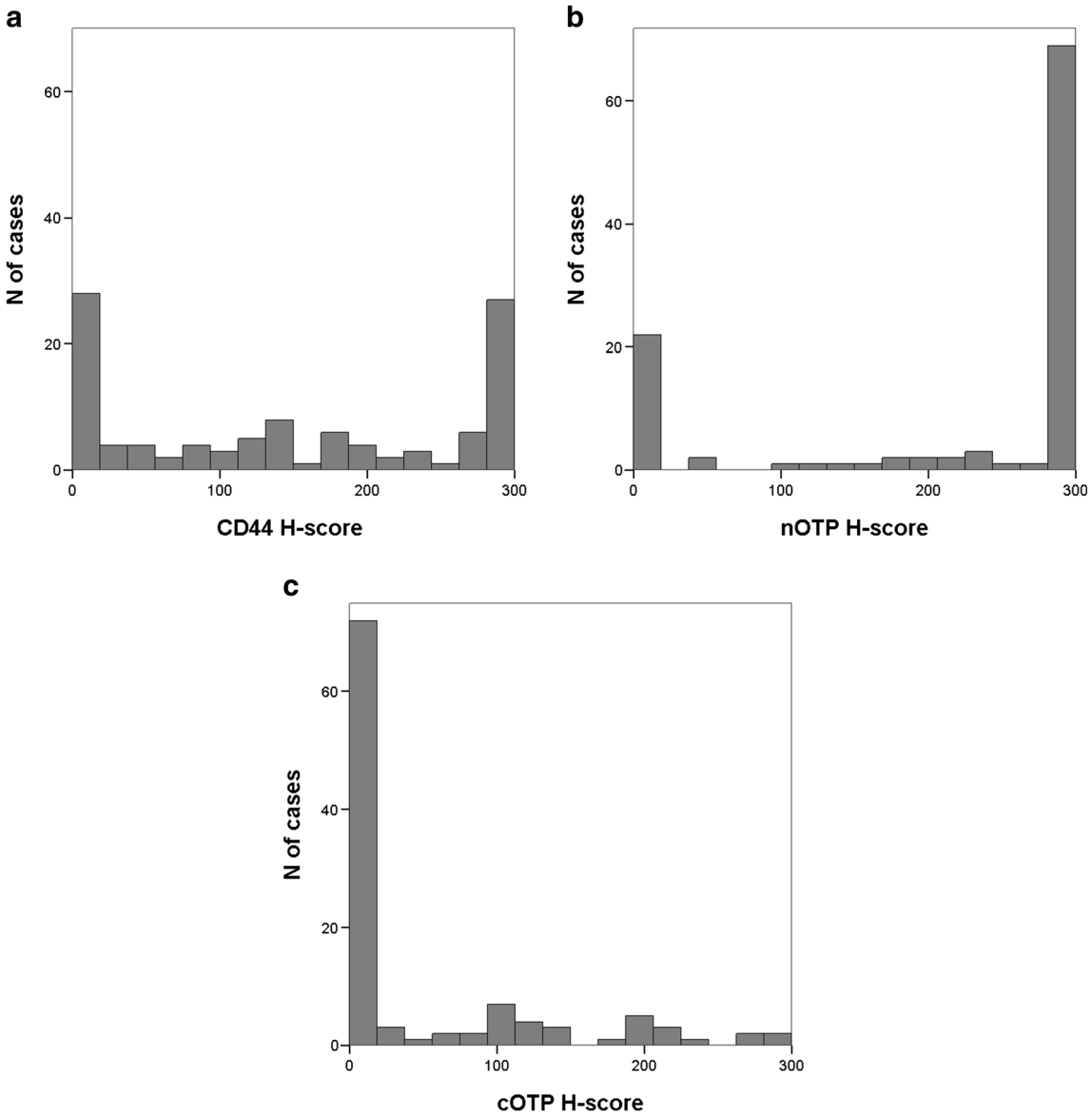

Fig. 1 Histograms showing the distribution of CD44 (**a**), nuclear (**b**) and cytoplasmic (**c**) OTP *H* scores

cOTP was not correlated with tumour Ki-67 index or with tumour stage, except a weak association of low cOTP expression level with early pathology stage.

Diagnostic Performance of CD44 and OTP to Discriminate Between TC vs AC

The highly significant association of CD44 and nOTP expression levels with histological subtype renders them potential useful diagnostic biomarkers to distinguish typical from atypical histological subtypes of PC. ROC curves (Fig. S4) showed that these two biomarkers had good but not excellent diagnostic accuracy with an area under the curve (AUC) of 0.801 (95 % CI = 0.703–0.899, $p < 0.001$) for CD44 and 0.757 (95 % CI = 0.646–0.868, $p < 0.001$) for nOTP. By contrast, cOTP was a non-diagnostic biomarker with no correlation to histological subtype and an AUC of 0.408 (95 % CI = 0.276–0.541, $p = 0.173$). Of note, the highest diagnostic performance was achieved by Ki-67 index with AUC of 0.950 (95 % CI = 0.910–0.991, $p < 0.001$).

Prognostic Significance of CD44 and OTP

In this study, 86 PC patients underwent radical surgical resections with a median follow-up period of 35 months (range, 8–153 months). In total, 13 patients (15.1 %) relapsed, 4 with TC and 9 with AC. Six patients relapsed at 8–27 months of follow-up (1 with TC, 5 with AC), 6 at 31–58 months (2 with TC, 4 with AC) and 1 with TC relapsed at 95 months. Two patients, both with AC, relapsed at the mediastinum and 11 relapsed at distant organs (systemic relapse). Median RFS of the study population was 95 months and 3-year RFS was 87 %. By histological subtype, 3-year RFS was 98 % for TC and 67 % for AC ($p < 0.001$), respectively.

Both CD44 and nOTP *H* scores were prognostic for RFS as continuous variables, with HR = 0.989 (95 % confidence intervals [CI] = 0.981–0.996, $p = 0.002$) and HR = 0.995 (95 % CI = 0.991–0.999, $p = 0.012$), respectively. By contrast, cOTP *H* score was not prognostic ($p = 0.193$). Due to the bimodal distribution of *H* score for both CD44 and nOTP, the central value was used as cutoff to classify protein expression as high (*H* score ≥150) or low (*H* score < 150). Patients with high CD44 and nOTP tumour expression had longer RFS with HR = 0.061 (95 % CI = 0.008–0.475, $p = 0.008$) and HR = 0.270 (95 % CI = 0.090–0.814, $p = 0.020$), respectively. Three-year RFS was better for patients with high CD44 (98 vs 76 %, $p < 0.001$) and nOTP expression (94 vs 70 %, $p = 0.013$), compared to those with low expression (Fig. 2).

Prognostic Significance of Combined Expression of CD44 and OTP

Notably, no patient with both high CD44 and nOTP protein expression relapsed within the follow-up period of this study. Three-year RFS of patients with combined high CD44 and nOTP expression was significantly better compared to those with low expression of one (3-year RFS = 82 %, $p < 0.001$) or both of the proteins (3-year RFS = 68 %, $p < 0.001$). In contrast, there was no statistically significant difference in RFS between patients with tumours characterised by low expression of one or both proteins ($p = 0.861$, Fig. 3). As such, high CD44 and high nOTP expression may delineate a favourable prognostic group in PC.

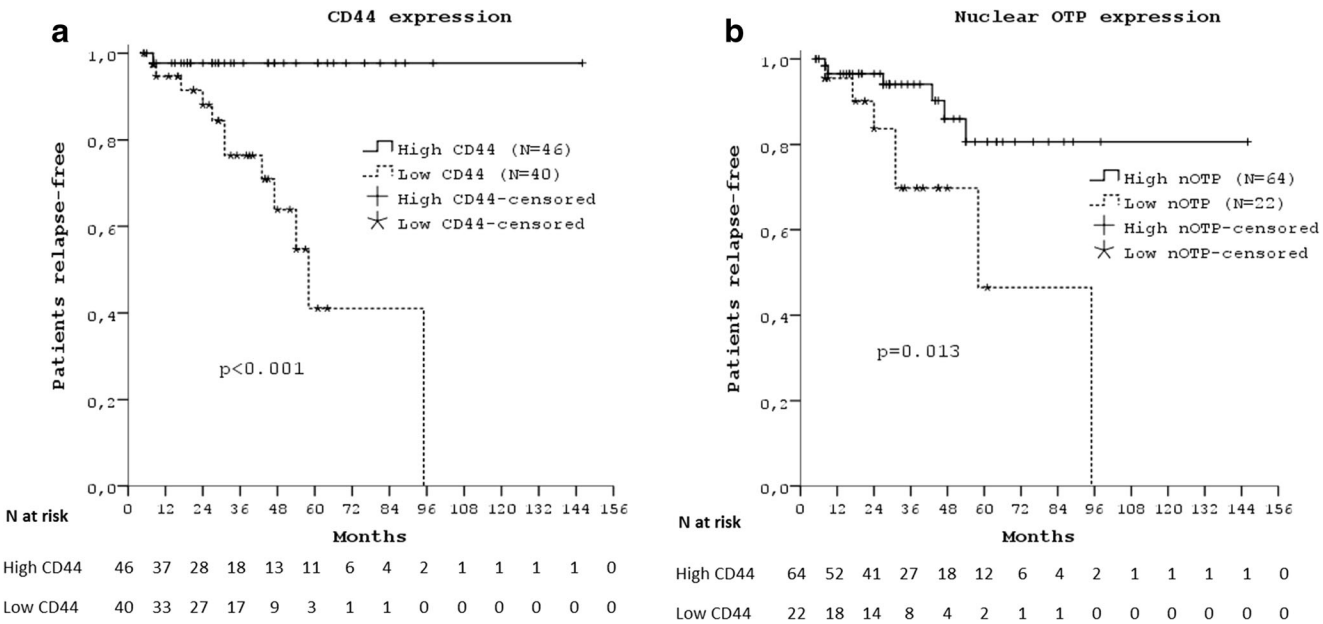

Fig. 2 Relapse-free survival curves according to CD44 (**a**) and nuclear OTP (**b**). *nOTP* nuclear OTP

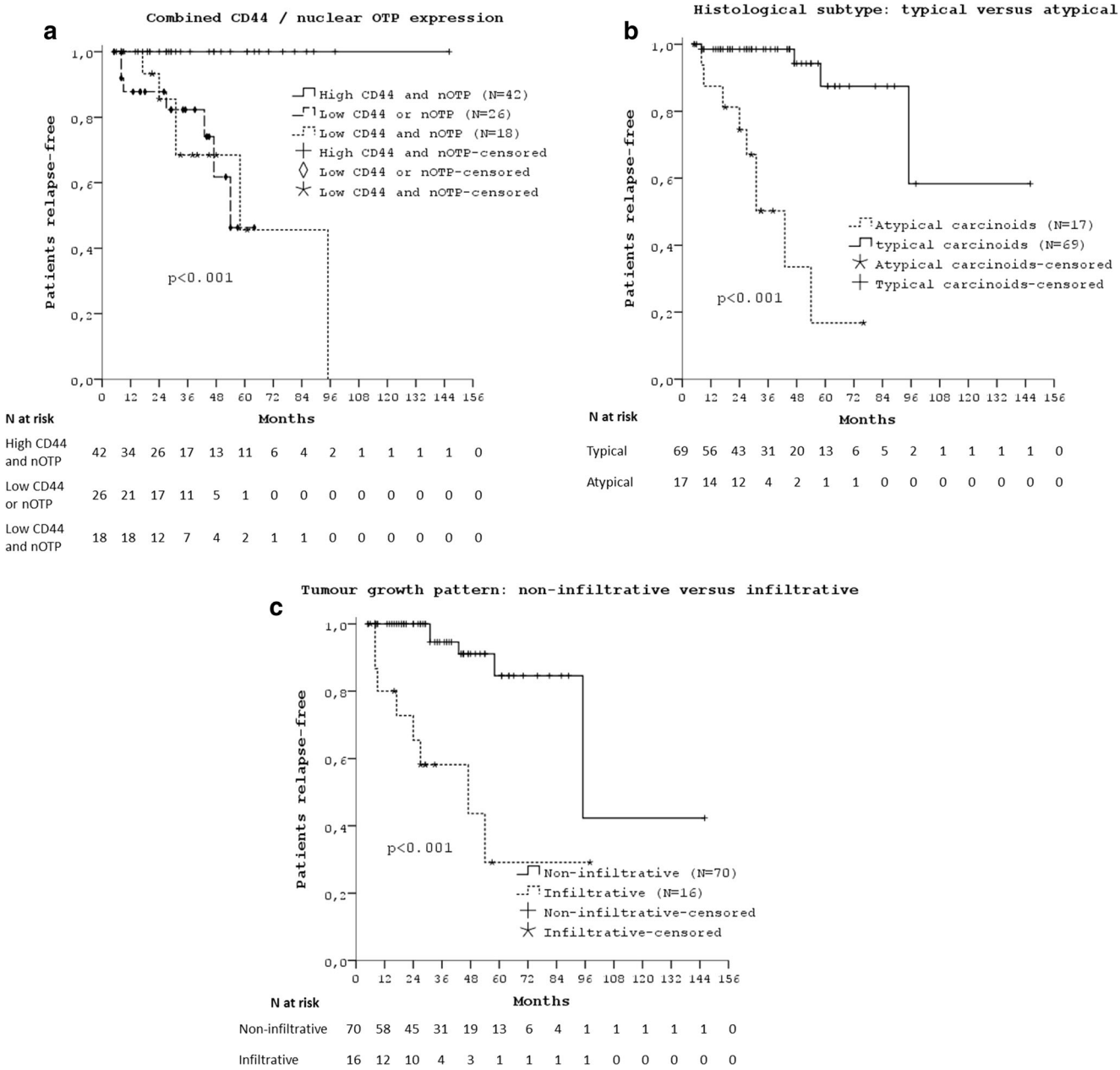

Fig. 3 Relapse-free survival curves according to combined CD44 and nuclear OTP expression (**a**), histological subtype (**b**) and tumour growth pattern (**c**)

Univariate analysis showed that high expression of both CD44 and nOTP, typical histology, non-infiltrative tumour growth pattern, Ki-67 index <5 % and early pathology stage (pT1N0M0) were prognostic for RFS (Table 3 and Fig. 3). In multivariate analysis, combined CD44/nOTP expression levels, histological subtype and the type of tumour growth pattern were independent prognostic factors for RFS (Table 4).

Subgroup analysis showed that combined high CD44 and high nOTP tumour expression was a favourable prognostic factor for RFS in patients with typical carcinoid and non-infiltrative growth pattern (Fig. 4). This result was replicated in tumours of locally advanced pathological stage (greater than pT1N0) and typical histology or non-infiltrative growth pattern (Fig. 4). The number of cases and relapses within specific subgroups according to pathological stage (early vs locally advanced), histological subtype (TC vs AC), combined CD44 and nOTP expression (both high vs one or both low) and tumour growth pattern (non-infiltrative vs infiltrative tumour margins) is described in Table S3.

Potential Role of CD44 and nOTP in Prognostic Classification of PC

Aiming to give a more practical insight in the above findings, and considering that the assessment of mitotic count and

Table 3 Univariate analysis of prognostic factors for relapse-free survival

Characteristic	Value	*N*	3-year RFS (%)	Log-rank *p* value
Age (years)	<60	42	91	0.271
	≥60	44	82	
Gender	Male	34	80	0.137
	Female	52	92	
PS (ECOG)	0	62	92	0.222
	1	24	71	
Smoking	No	44	90	0.149
	Ex	25	65	
	Current	17	93	
Pathology stage	Early	38	97	**0.016**
	Locally advanced	48	80	
Resection margins	Clear (R0)	71	89	0.765
	Infiltrated (R1)	15	75	
Location	Central	53	89	0.250
	Peripheral	33	85	
Histology	Typical	69	98	**<0.001**
	Atypical	17	67	
Ki-67 index	<5 %	66	95	**<0.001**
	≥5 %	20	65	
TTF-1	Positive	37	86	0.269
	Negative	49	89	
Tumour growth pattern	Non-infiltrative	70	95	**<0.001**
	Infiltrative pattern	16	58	
DIPNECH	Absent	82	87	0.530
	Present	4	100	
CD44/nuclear OTP	Both highly expressed	42	100	**<0.001**
	One highly expressed	26	82	
	Both poorly expressed	18	68	

Characteristics of prognostic significance are shown in bold

DIPNECH diffuse pulmonary neuroendocrine hyperplasia, *Early stage* pathological tumour stage pT1N0, *Locally advanced stage* pathological tumour stage more advanced than pT1N0, *PS(ECOG)* performance status according to the Eastern Cooperative Oncology Group scale

necrosis is characterised by high interobserver variability, we examined the prognostic significance of CD44/nOTP for RFS in multivariate Cox-regression analysis excluding the WHO classification of PCs. Multivariate analysis indicated that only CD44/nOTP expression levels and tumour growth pattern were independent prognostic parameters (Table S4). We then classified cases in three groups: those with excellent prognosis characterised by high CD44 and nOTP expression, those with intermediate prognosis showing low CD44 and/or nOTP expression and non-infiltrative growth pattern and, finally, those with poor prognosis demonstrating low CD44 and/or nOTP expression and infiltrative growth pattern (Fig. 5). Notably, the *c* index for this classification was very high (0.902, 95 % CI = 0.884–0.918), statistically significantly higher ($p < 0.001$) than the *c* index for the WHO standard classification (0.811, 95 % CI = 0.788–0.834). Also interestingly, Ki-67 levels were not significantly different between the favourable (median Ki-67 = 1.8, 95 % CI = 1.3–2.3) and the intermediate prognosis group (median Ki-67 = 1.3, 95 % CI = 0.6–2.0, $p = 0.386$), as shown in Fig. S5. In contrast, Ki-67 levels were significantly higher in the poor prognosis group (median Ki-67 = 5.2, 95 % CI = 4.2–6.3, $p \leq 0.011$).

Table 4 Multivariate analysis of prognostic factors for relapse-free survival

Characteristic	Value	HR (95 % CI)	*p* value
Histology	Typical vs atypical	0.143 (0.027–0.742)	<0.001
Tumour growth pattern	Non-infiltrative vs infiltrative	0.090 (0.018–0.444)	<0.001
CD44/nuclear OTP	Both vs one vs none highly expressed	0.189 (0.063–0.566)	<0.001

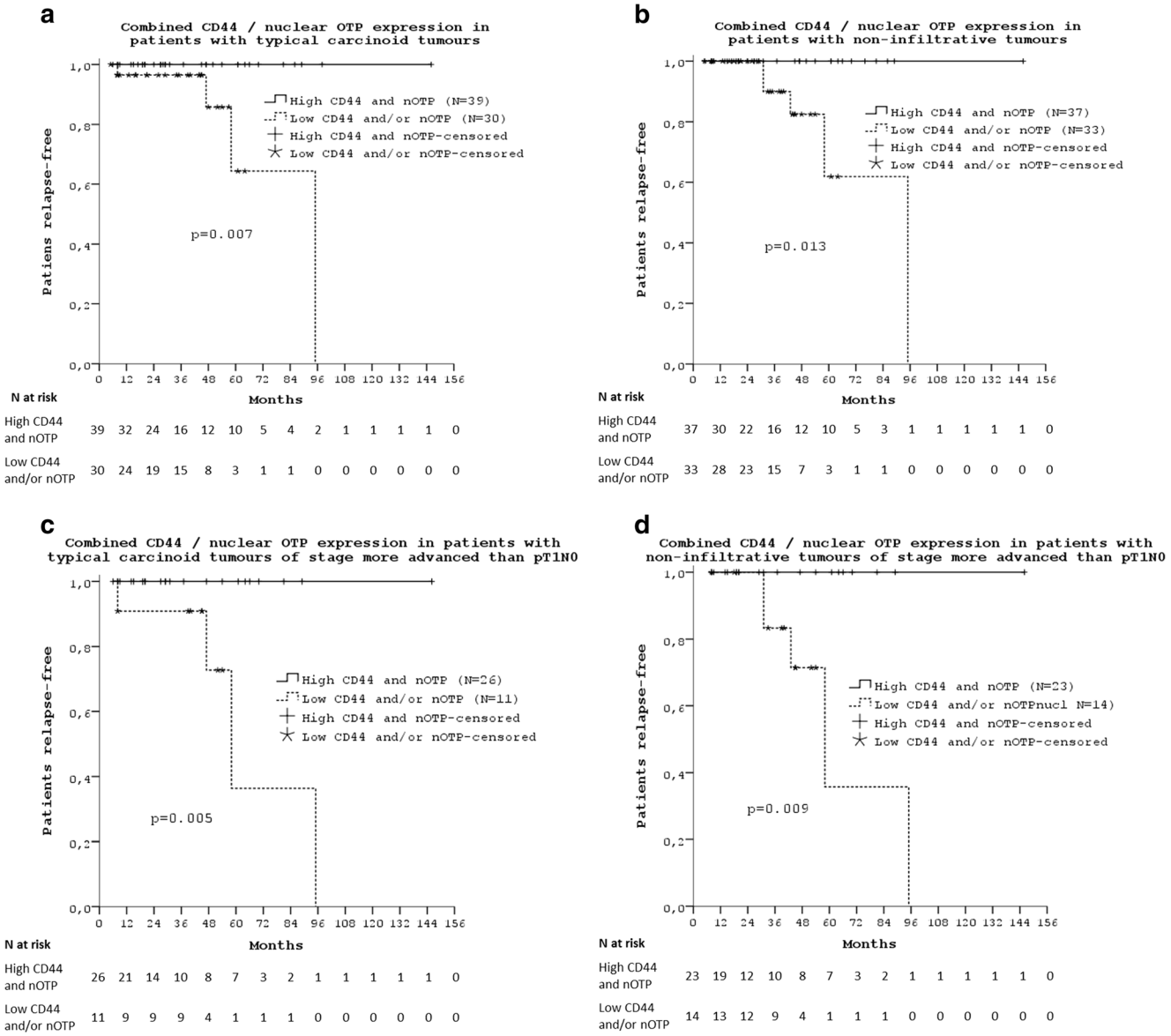

Fig. 4 Relapse-free survival curves according to combined CD44 and nuclear OTP expression in the patients subgroups with typical carcinoids (**a**), with non-infiltrative tumours (**b**), stage >pT1N0 and typical carcinoids (**c**) or stage >pT1N0 and non-infiltrative tumours (**d**)

Discussion

In the present study, we demonstrated that CD44 and nOTP are highly significant prognostic biomarkers of patient outcome in typical PC. CD44 and nOTP expression alongside the well-established WHO classification of PC may therefore aid in clinical decision making in the management of PC.

CD44, a cell surface receptor binding to extracellular matrix hyaluronan, has been extensively studied across multiple tumour types. CD44 mediates cancer cell survival, proliferation and motility, as well as the modulation of tumour microenvironment [9, 13]. In support of our data, two studies have demonstrated CD44 expression is gradually decreased from TC to AC and finally to very low levels in LCNEC and SCLC [14, 15].

Several studies have examined the prognostic role of CD44; however, results to date have been contradictory [9]. One explanation for this disparity may be the variable expression of CD44 isoforms with tumour subtype and site [9]. An alternative technical explanation is that different studies utilised different anti-CD44 antibody and so detected different CD44 isoforms. In one study of TC, standard CD44 and variants 7–8 and 9 were associated with increased OS [7]. In more recent work using fresh frozen tissue from a large cohort of early and advanced PC, standard CD44 expression was a strong prognostic factor for OS in all PC patients but also in the AC subgroup [8].

OTP is a member of the homeobox protein family and has a well-defined role in embryonic neurodevelopment [10]. Our group has demonstrated [16] that in adult life OTP expression

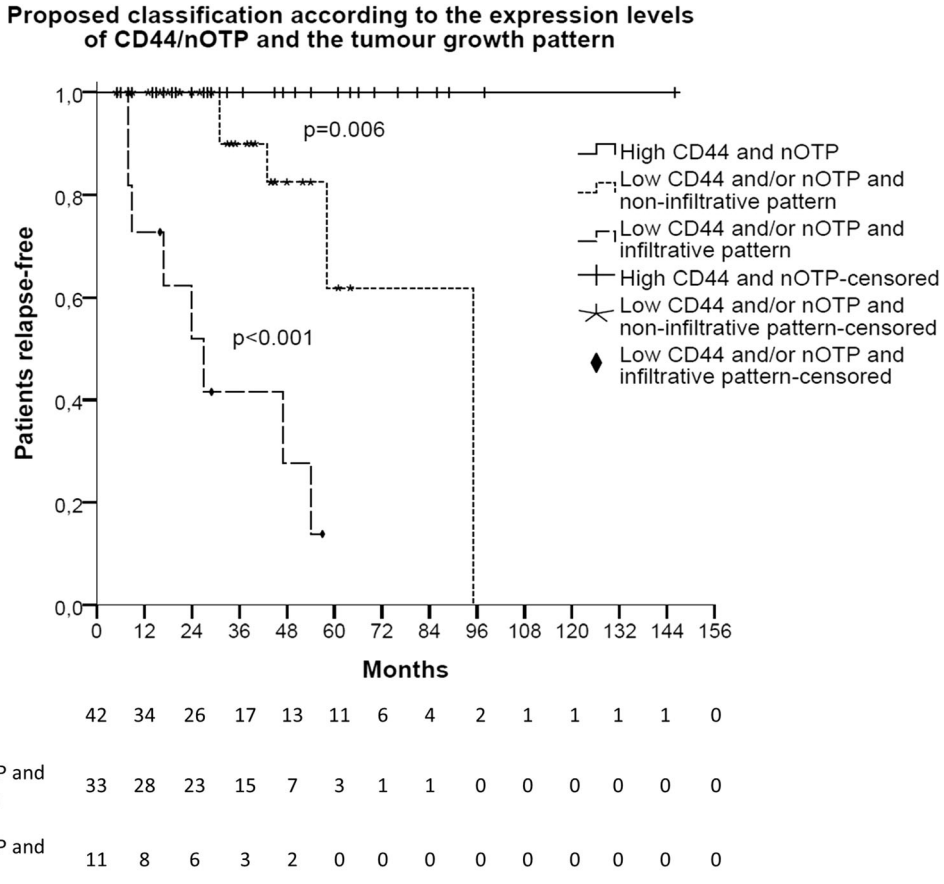

Fig. 5 Classification of tumours in three prognostically relevant groups according to the tumour growth pattern and the expression levels of CD44/nOTP. Log-rank test showed that relapse-free survival (*RFS*) in the group with high levels of both CD44 and nOTP was statistically longer compared with those with non-infiltrative tumour growth pattern and low levels of CD44 and/or nOTP ($p = 0.006$) and infiltrative tumour growth pattern and low levels of CD44 and/or nOTP ($p < 0.001$). Also, those with non-infiltrative tumour growth pattern and low levels of CD44 and/or nOTP had significantly longer RFS compared to those with infiltrative tumour growth pattern and low levels of CD44 and/or nOTP ($p < 0.001$)

is restricted to PC and is only very rarely found in normal tissue or other solid cancers. OTP is thus a useful diagnostic biomarker in PC. In the current study, a gradient of expression of both OTP and CD44 was shown between TC and AC. Due to this overlap, these proteins are unsuitable as diagnostic markers to distinguish TC from AC. However, in their published work, Swarts et al. showed that OTP added prognostic value in cases of PC that are difficult to categorise into TC or AC [17]. This indicated a role for OTP as a prognostic biomarker which might be used alongside standard histological subtyping according to mitotic index, necrosis and Ki-67, to characterise the prognosis of PC cases.

Although there is strong evidence from previous studies that CD44 and OTP are prognostic for patients with lung carcinoids [7, 8], potential bias may restrict their robustness as biomarkers. The recent recruitment period of this study (2003–2015) was a weakness as it restricted the sample size and the length of follow-up, which may lead to the underdetection of late relapses, especially in TC cases. However, in studies including older cohorts, the difference in the quality of FFPE specimens and imaging methods across different time periods, the evolution of surgical techniques and experience and the variability in the follow-up policy may also greatly increase data heterogeneity, making it difficult to interpret the results. Selection of OS as endpoint risks increasing bias due to the heterogeneity of treatment regimens post-relapse and the inclusion of non-cancer-related deaths. In the present study, we carefully selected 86 homogeneously treated patients for prognostic analysis. All operations were radical and were performed by a single surgeon, all patients were followed up in the same institution and adequate data were available for each study participant. Additionally, RFS was chosen as endpoint to prevent any bias from treatment post-relapse. Expression of both CD44 and nOTP was recorded using a standard *H* score, intending to reinforce the reproducibility of our data.

The present study validated the prognostic role of CD44 and nOTP. Combined high CD44 and nOTP expression was a strong independent prognostic factor for RFS, along with histological subtype and the tumour growth pattern. This was

confirmed in TC patients with locally advanced pathology stage. By contrast, early pathology stage TC patients did not relapse, irrespective of CD44-nOTP expression. Neither CD44 nor nOTP was correlated with Ki-67 or mitotic index.

The significance of tumour growth pattern is less well described. Infiltrative growth pattern is a poor prognostic factor across multiple tumour types [18–23]. To our knowledge, no previous studies showed the prognostic significance of tumour growth pattern [24, 25]. However, our results will need to be validated by further studies.

The current study proposes the use of combined CD44-nOTP expression for the prognostic classification of TC, especially those with stage >pT1N0. Twenty-six TC cases (38 % of total TC) with combined high CD44-nOTP expression and stage >pT1N0 might be better allocated in the low-risk TC patients together the 32 TC patients (46 %) with stage pT1N0. In this way, only 11 TC patients (16 %) still remained in the high-risk group requiring more intense clinical and radiological follow-up, thus significantly improving health costs and decreasing unnecessary exposure to radiation. However, prolonged follow-up might still be needed to detect potential late relapses.

Although classification of PC into TC and AC has strong prognostic impact, accumulating evidence indicates significant limitations of the WHO system, mainly poor interobserver concordance especially in the assessment of mitotic count [17, 26] and technical difficulties when assessing small biopsies. We described for the first time a new classification system combining the tumour growth pattern and the expression levels of CD44/nOTP. Patients with tumours showing infiltrative tumour growth pattern and low expression of CD44 and/or nOTP demonstrated extremely poor outcome. Notably, this classification showed higher prognostic accuracy compared with the WHO system, which is reflected on the high *c* index. Nevertheless, this finding needs validation in a separate cohort.

In conclusion, the present study showed that CD44/nOTP expression was an independent predictor of RFS in patients with radically operated PC tumours. The above results demonstrate practical suggestions on how prognostic stratification of PC patients can be improved by combining CD44/nOTP expression levels and the tumour growth pattern.

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Compliance with Ethical Standards

Conflict of Interest All the authors declare that they have no conflict of interest.

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