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ORIGINAL ARTICLE

Oncocytic and spindle cell typical carcinoids of lung: different immunophenotype and biological behavior

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Abstract

Pulmonary typical carcinoids (TCs) are uncommon, well-differentiated neuroendocrine tumors of the lung that do not exhibit necrosis and have fewer than two mitoses per 2 mm^2, as defined by the current World Health Organization classifications. Despite their low-grade status and favorable prognostic impact, the protein expression profile and morphological characteristics associated with tumor progression and metastatic spread remain largely unidentified. Oncocytic and spindle cell histological variants are acknowledged for their role in differential diagnosis, though their clinical significance remains a topic of debate. We centrally reviewed a multicenter series of 297 TCs to identify cases of oncocytic and spindle cell variants. We examined associations with clinicopathological features and immunohistochemical markers (orthopedia homeobox protein, thyroid transcription factor 1, mammalian achaete-scute homologue 1, somatostatin receptor 2A, Ki-67, anti-mitochondria, and S100); these data were further related to disease-free survival (DFS), overall survival, and cancer-specific survival (CSS). Our analysis identified oncocytic TCs ($n = 36$, 12.1%), spindle cell TCs ($n = 55$, 18.5%), and ordinary TCs, defined as those without either variant or with variants that were not prominent ($n = 206$, 69.4%). Interestingly, ordinary tumors were associated with a higher number of tumor-related deaths ($p = 0.01$) compared to the other histological variants. Additionally, patients with spindle cell morphology had longer CSS compared to those with ordinary morphology ($p = 0.04$). Parameters such as histological variant, age, tumor stage, and Ki-67 were significantly linked to DFS on multivariable analysis, even after accounting for differences between centers.

In conclusion, oncocytic, spindle cell, and ordinary TCs are linked to distinct clinicopathological characteristics and exhibit varying clinical outcomes.

Keywords: typical carcinoid; oncocytic cell; spindle cell; immunohistochemistry

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No conflicts of interest were declared.

Introduction

The 2021 WHO Classification of Thoracic Tumours (WHO-TT 2021) and the 2022 WHO Classification of Endocrine and Neuroendocrine Tumors define typical carcinoids (TCs) as rare well-differentiated lung neuroendocrine neoplasms (LNENs) that do not exhibit necrosis and have fewer than two mitoses per 2 mm^2 [1,2]. The well-known typical morphologic features of TCs are similar to well-differentiated neuroendocrine tumors (NETs) of other sites. Well-differentiated pulmonary carcinoids are typically characterized by uniform round nuclei and/or with plasmacytoid cytology, sleek nuclear membranes, salt and pepper chromatin, inconspicuous nucleoli, and different ordinary architectural growth pattern (trabecular, organoid, nesting, cordiform, and/or rosettes) [3]. TCs can exhibit a staggering number of variant morphologies including the spindle variant, which is particularly common in peripherally localized TCs and can simulate other specific spindle cell neoplasms [4]. Oncocytic features can also cause diagnostic challenges [4]: TCs entirely composed of oncocytic cells are rather rare, although areas of an oncocytic component can be generally seen in these tumors [5,6]. Most of the previously described cases of oncocytic neuroendocrine tumors (ONTs) have been well-differentiated neoplasms; on the contrary no oncocytic poorly differentiated counterparts have been documented [7]. Currently, oncocytic and spindle cell histological variants are recognized for their role in differential diagnosis but their clinical significance continues to be widely debated, likely due to their rarity and biological heterogeneity. The present study aims to recognize clinicopathological features of oncocytic and spindle cell histological variants of TCs and to examine their correlation with immunohistochemical (IHC) markers, including thyroid transcription factor 1 (TTF-1), orthopedia homeobox protein (OTP), mammalian achaete-scute homologue 1 (Ascl-1), somatostatin receptor 2A (SSTR-2A), Ki-67, anti-mitochondria, and S100 protein, and with clinical outcome.

Materials and methods

Study design and case selection

We carried out a comprehensive retrospective analysis, related to the period 1988–2018, from the archives of two major cancer centers in Italy: IRCCS National Cancer Institute (INT) in Milan and ASST Spedali Civili in Brescia. All patients with one of these specific histologic diagnoses were selected: 'typical lung carcinoid', 'lung carcinoid tumor', 'peripheral carcinoid', 'bronchial carcinoid', 'spindle cell carcinoid', 'oncocytic carcinoid', or with 'oncocytic features' or 'oncocytic changes'. Conversely, the following criteria were not expressly evaluated in the present series: (1) cases that were not treated with surgery intended to be curative; (2) poorly differentiated neuroendocrine components; (3) biopsy-only available; (4) cases of unknown primary site origin; (5) diffuse idiopathic pulmonary neuroendocrine cell hyperplasia and neuroendocrine hyperplasia. A total of 297 TCs were identified. The clinical standards of the 1975 and 1983 Declaration of Helsinki and the Ethics Committee of Fondazione IRCCS INT (No. INT 171/16) were respected. Before inclusion in the study, two specialized pathologists (MM and CC) centrally reviewed all histologic diagnoses, blinded to clinical and follow-up information. Morphologic profile and identification of TCs were dependent on analysis of three consecutive sections from representative formalin-fixed, paraffin-embedded blocks stained with hematoxylin–eosin (H&E) and for the main two neuroendocrine markers synaptophysin (Syn) and chromogranin A (CgA). Overall, a total of 297 cases respected the morphologic criteria for carcinoids according to the current WHO classifications, the 2021 Classification of Thoracic Tumours and the 2022 Classification of Endocrine and Neuroendocrine Tumors, and were included in the study [1,2].

Histologic analysis and immunohistochemistry

Morphological inclusion criteria were: (1) well-differentiated neuroendocrine morphology; (2) ordinary architectural

growth pattern of the tumor (trabecular, organoid, nesting, cordiform, and/or rosettes); (3) spindle cell pattern (in at least 20% of the tumor); (4) oncocytic pattern (in at least 20% of the tumor) (Figure 1); (5) mitosis in hot-spot fields according the WHO guidelines 2021 and 2022 [1,2]; (6) evaluation of necrotic areas; (7) stage and grading according to the Union for International Cancer Control/American Joint Committee on Cancer 8th edition; (8) vascular and/or perineural invasion; (9) intra and/or peritumoral lymphocyte infiltrate; (10) microscopic invasion of pleura and/or bronchus; and (11) tumor spread in air spaces.

The two major neuroendocrine IHC markers (Syn and CgA) were used in order to demonstrated neuroendocrine origin and differentiation; according to the 2022 WHO Classification of Endocrine and Neuroendocrine Tumors and the 2019 WHO Digestive System Tumours, the Ki-67 labeling index was calculated by using the MIB antibody to measure the percentage of positive cells among 500–2,000 tumor cells counted in areas with the most intense nuclear staining ('hot spots'); anti-mitochondria antibody was included to highlight oncocytic component (Figure 2A); S100 protein was stained to underline sustentacular cells in spindle-cell components (Figure 2B); OTP, TTF1, Ascl-1, and SSTR-2A were also performed (supplementary material, Table S1 shows their respective antibodies). To better limit heterogeneity in the evaluation, all IHC antibodies were classified as positive irrespective of the proportion of immunoreactivity but only nuclear staining was accepted. The only exception was SSTR-2A staining, which was instead assessed using the semiquantitative two-tiered system score by Volante *et al*; negative for scores of 0 and 1 (respectively, absence of immunoreactivity and/or pure cytoplasmic immunoreactivity) and positive for 2 and 3 positivity (circumferential membranous reactivity in less and/or in more than 50% of tumor cells, irrespective of the presence of cytoplasmic staining) [8].

Statistical analysis

Data were examined using descriptive statistical methods. The Fisher's exact test was used for correlations between clinicopathological factors, demographic features, and morphological groups (ordinary cells, oncocytic cells, spindle cells). Overall survival (OS) and cancer-specific survival (CSS) were evaluated from the diagnosis date to the death date from any cause or death specifically related to the tumor, respectively. Disease-free survival (DFS) was estimated, only in stage I–II–III patients, from the date of diagnosis until the occurrence of the first relapse, tumor-related death, or the final follow-up. The Kaplan–Meier method was used to generate OS and DFS curves. The log-rank test was employed to evaluate the differences in survival between patient groups. Multivariable and univariable Cox proportional hazards models were utilized to examine the relationship between clinicopathological features and OS, CSS, and DFS. Manual backward elimination was employed to identify the optimal set of predictors, focusing on clinically important criteria. Hazard ratios (HR) are provided along with their corresponding 95% confidence intervals (CI). Analysis of data was conducted through the R software setting

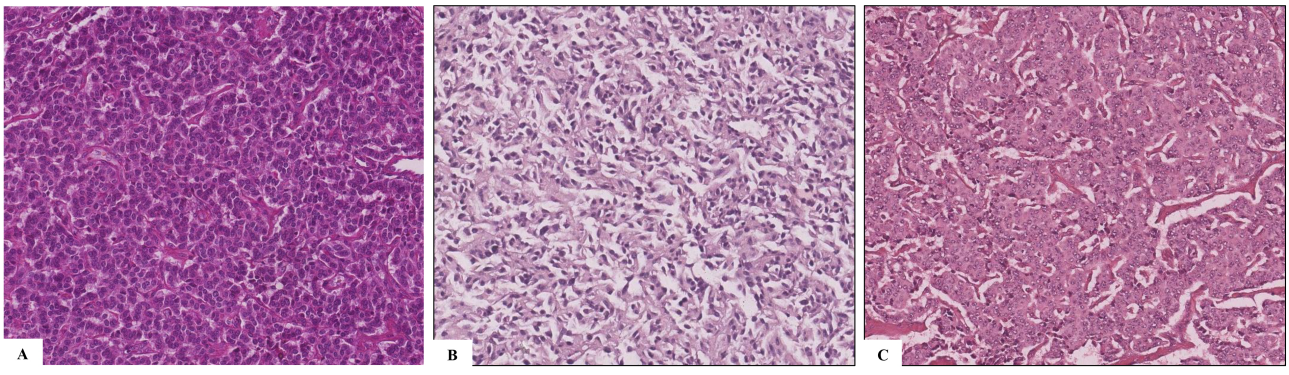

Figure 1. According to the current WHO classifications, lung typical carcinoid tumor can exhibit canonical trabecular/organoid growth pattern or noncanonical histological variant morphologies. (A) Canonical pattern with uniform tumor cells, polygonal shape, round-to-oval nuclei with salt and pepper chromatin, as well as inconspicuous nucleoli and moderate to abundant eosinophilic cytoplasm. (B) Spindle cell variant with fascicles of plump spindle cells separated by thin fibrovascular septa, and (C) oncocytic variant with organoid pattern and tumor cells with an ample amount of granular oncocytic cytoplasm, round-to-oval nuclei, and coarse chromatin. Magnification, ×20.

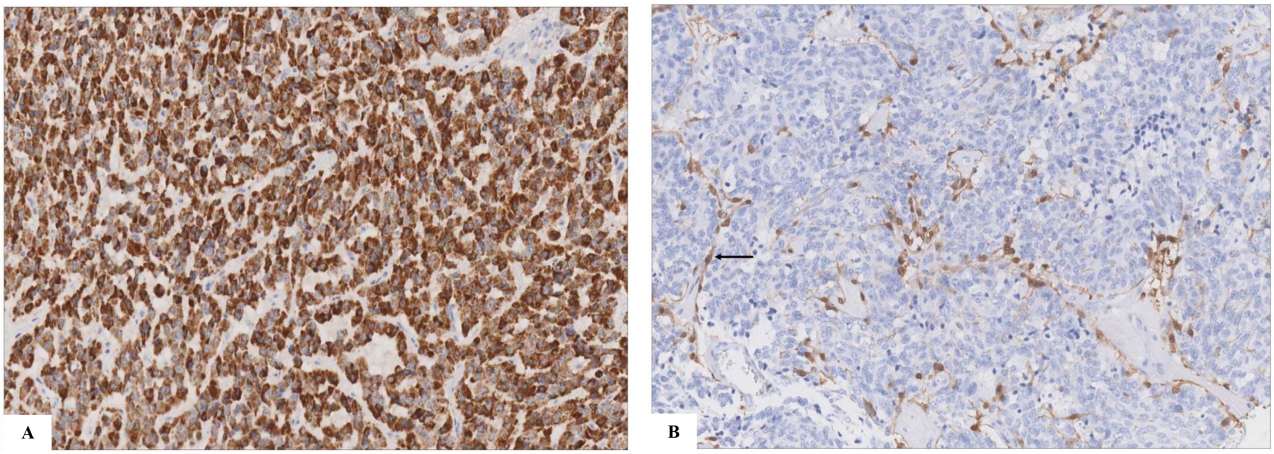

Figure 2. (A) Anti-mitochondrial antibody positivity was widely and homogeneously evident in the cytoplasm of the neoplastic cells of oncocytic typical carcinoid; (B) S100 protein expression in well-recognized stellate-shaped sustentacular cells (arrow) in a spindle-cell typical carcinoid; Magnification, ×20.

for statistical analysis and graphical representation (R Foundation, 4.0.3 version, Austria, Vienna). All tests were conducted as two-sided, and p values less than 0.05 were deemed statistically significant.

Results

Morphologic and clinicopathological features

Table 1 shows the main clinicopathological characteristics of the 297 TC patients included in the study. Overall, pathologic review identified oncocytic ($n = 36$), spindle cell ($n = 55$), and ordinary ($n = 206$) TCs. The entire cohort had a higher proportion of females compared to males (63.3% versus 36.7%), with a median age of 60 years. The series included 235 (79.1%) stage I, 36 (12.1%) stage II, 21 stage III (7.1%), and 5 (1.7%) stage IV tumors. Overall, a slight majority of the patients were never smokers ($n = 86$, 51.8%), which was also the case in tumors with oncocytic and spindle cell features. Surgical treatment included 92 (30.3%) partial resections, 182 (61.3%) lobectomies, and 23 (7.7%) bilobectomies or pneumonectomies.

Spindle cell TCs were associated with peripheral location ($n = 37$, 67.3%, $p < 0.001$), partial surgical resections ($n = 29$, 52.7%, $p < 0.0009$), insular/solid morphological pattern ($n = 28$, 50.9%, $p <$ 0,001), and favorable outcome ($p = 0.01$). Oncocytic TCs were found to be associated with a central location ($n = 29$, 80.6%, $p < 0.001$), early age at diagnosis (<50 versus ≥50 years, $p < 0.001$), organoid/nesting/trabecular morphological pattern ($n = 22$, 61.1%, $p <$ 0,001), and bronchial microscopic invasion ($n = 21$, 65.6%, $p = 0.006$).

Interestingly, ordinary TCs showed more tumor associated deaths ($n = 31$, 15.0%, $p = 0.01$) than other nonordinary histological variants.

Furthermore, selected oncocytic TCs ($n = 36$) and spindle cell TCs ($n = 55$) were in turn subdivided into two groups according to moderate (20–50%) and predominant (>50%) oncocytic or spindle cell component, respectively. The same associations with clinicopathological features (supplementary material, Tables S2, S3) and IHC markers (supplementary material, Tables S4, S5) were evaluated. Based on spindle cell morphology, the results highlighted a stronger association between TCs with a predominant spindle cell component (>50%) and peripheral location ($n = 19$, 82.6%, $p = 0.05$) (supplementary material, Table S3).

With regard to oncocytic cell component, no statistically significant results were obtained by comparing tumors with predominant to those with moderate oncocytic component.

IHC markers

Table 2 shows the distribution of examined IHC markers. Spindle cell TCs were associated with absence of SSTR-2A-IR ($n = 35$, 76.1%, $p < 0.0001$) but presence of TTF-1-IR ($n = 11$, 23.9%, $p = 0.04$), OTP-IR ($n = 41$, 91.1%, $p = 0.03$), Ascl-1-IR ($n = 22$, 47.8%, $p = 0.0001$), and S100 protein expressed in sustentacular cells ($n = 27$, 62.8%, $p < 0.0001$) (Figure 3A–E).

On the contrary, oncocytic TCs showed associations with absence of TTF-1-IR ($n = 33$, 91.7%, $p = 0.04$)

Table 1. Characteristics of patients with typical carcinoids according to cell morphology

	All patients	Canonical TCs	Oncocytic cells TCs	Spindle cells TCs	p^*
Total	297 (100)	206 (100)	36 (100)	55 (100)	
Gender					
Female	188 (63.3)	125 (60.7)	21 (58.3)	42 (76.4)	0.07
Male	109 (36.7)	81 (39.3)	15 (41.7)	13 (23.6)	
Age					
<50 years	90 (30.3)	64 (31.1)	20 (55.6)	6 (10.9)	**0.0005**
50–59 years	58 (19.5)	35 (17.0)	6 (16.7)	17 (30.9)	
60–69 years	92 (31.0)	63 (30.6)	7 (19.4)	22 (40.0)	
70+ years	57 (19.2)	44 (21.3)	3 (8.3)	10 (18.2)	
Stage					
I	235 (79.1)	167 (81.1)	22 (61.1)	46 (83.6)	**0.05**
II	36 (12.1)	24 (11.7)	9 (25.0)	3 (5.5)	
III	21 (7.1)	12 (5.8)	4 (11.1)	5 (9.1)	
IV	5 (1.7)	3 (1.4)	1 (2.8)	1 (1.8)	
Smoking					
Never smoker	86 (51.8)	49 (49.5)	16 (53.3)	21 (56.8)	0.5
Former smoker	38 (22.9)	25 (25.3)	4 (13.3)	9 (24.3)	
Current smoker	42 (25.3)	25 (25.3)	10 (33.3)	7 (18.9)	
Mitoses					
0	186 (62.6)	142 (68.9)	17 (47.2)	27 (49.1)	**0.003**
1	111 (37.4)	64 (31.1)	19 (52.8)	28 (50.9)	
Location					
Central	190 (64.0)	143 (69.4)	29 (80.6)	18 (32.7)	**<0.0001**
Peripheral	107 (36.0)	63 (30.6)	7 (19.4)	37 (67.3)	
Vascular invasion					
Absent	225 (81.5)	158 (81.9)	22 (66.7)	45 (90.0)	**0.04**
Present	51 (18.5)	35 (18.1)	11 (33.3)	5 (10.0)	
Perineural invasion					
Absent	258 (93.8)	179 (93.2)	30 (90.9)	49 (98.0)	0.4
Present	17 (6.2)	13 (6.8)	3 (9.1)	1 (2.0)	
Intratumoral lymphocyte infiltrate					
Absent	228 (88.9)	162 (82.2)	27 (79.4)	39 (76.5)	0.6
Present	54 (19.1)	35 (17.8)	7 (20.6)	12 (23.5)	
Peritumoral lymphocyte infiltrate					
Absent	237 (82.9)	167 (84.8)	30 (85.7)	40 (74.1)	0.2
Present	49 (17.1)	30 (15.2)	5 (14.3)	14 (25.9)	
Microscopic invasion					
Absent	99 (35.7)	75 (38.7)	5 (15.6)	19 (37.3)	**0.006**
Positive STAS	32 (11.6)	21 (10.8)	1 (3.1)	10 (19.6)	
Bronchus	127 (45.8)	88 (45.4)	21 (65.6)	18 (35.3)	
Pleura	19 (6.9)	10 (5.2)	5 (15.6)	4 (7.8)	
Morphological pattern					
Insular/solid	85 (28.9)	45 (22.2)	12 (33.3)	28 (50.9)	**0.0004**
Trabecular/nested/organoid	202 (68.7)	154 (75.8)	22 (61.1)	26 (47.3)	
Other	7 (2.4)	4 (2.0)	2 (5.6)	1 (1.8)	
Residual tumor					
R0	224 (91.8)	155 (92.8)	28 (96.5)	41 (85.4)	0.2
R1-R2	20 (8.2)	12 (7.2)	1 (3.5)	7 (14.6)	
Surgery					
Lobectomy	182 (61.3)	132 (64.1)	25 (69.4)	25 (45.5)	**0.0009**
Bilobectomy/pneumonectomy	23 (7.7)	17 (8.3)	5 (13.9)	1 (1.8)	
Partial resection	92 (30.3)	57 (27.7)	6 (16.7)	29 (52.7)	
Tumor-associated deaths					
No	261 (87.9)	175 (85.0)	32 (88.9)	54 (98.2)	**0.01**
Yes	36 (12.1)	31 (15.0)	4 (11.1)	1 (1.8)	

Note: Significant *p* values are shown in bold font.
STAS, spread through air spaces; TCs, typical carcinoids.
*p value based on the Fisher's exact for categorical variables.

Table 2. Immunohistochemical markers of patients with typical carcinoids according to cell morphology

	All patients	Canonical TCs	Oncocytic cells TCs	Spindle cells TCs	p*
Ki-67 Index					
Ki-67 < 3%	248 (95.8)	164 (94.3)	33 (100)	51 (98.1)	0.4
Ki-67 ≥ 3%	11 (4.2)	10 (5.7)	0 (0.0)	1 (1.9)	
TTF-1					
Absent	228 (88.0)	160 (90.4)	33 (91.7)	35 (76.1)	**0.04**
Present	31 (12.0)	17 (9.6)	3 (8.3)	11 (23.9)	
SSTR-2A					
Absent	95 (36.8)	54 (30.7)	6 (16.7)	35 (76.1)	**<0.0001**
Present	163 (63.2)	122 (69.3)	30 (83.3)	11 (23.9)	
OTP					
Absent	57 (22.5)	42 (24.4)	11 (30.6)	4 (8.9)	**0.03**
Present	196 (77.5)	130 (75.6)	25 (69.4)	41 (91.1)	
Ascl-1					
Absent	191 (74.6)	134 (77.0)	33 (91.7)	24 (52.2)	**0.0001**
Present	65 (25.4)	40 (23.0)	3 (8.3)	22 (47.8)	
Anti-mitochondria					
Absent	142 (61.5)	108 (70.6)	0 (0.0)	34 (80.1)	–
Present	89 (38.5)	45 (29.4)	36 (100)	8 (19.1)	
S100					
Absent	159 (69.7)	111 (74.0)	32 (91.4)	16 (37.2)	**<0.0001**
Present	69 (30.3)	39 (26.0)	3 (8.6)	27 (62.8)	

Note: Significant *p* values are shown in bold font.
Ascl-1, mammalian achaete-scute homolog 1; OTP, orthopedia homeobox protein; SSTR-2A, somatostatin receptor 2A; TCs, typical carcinoids; TTF-1, thyroid transcription factor 1.
*p value based on the Fisher's exact test for categorical variables.

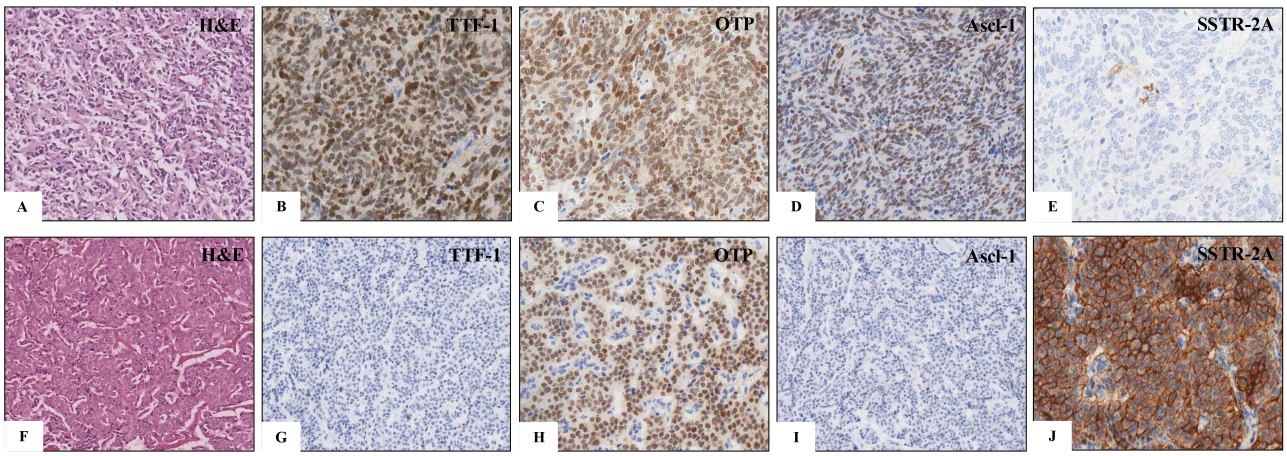

Figure 3. (A) Spindle cell typical carcinoid and (F) oncocytic typical carcinoid morphological and immunohistochemical profile based on (B, G) TTF-1, (C, H) OTP, (D, I) Ascl-1, and (E, J) SSTR-2A expression. Magnification, ×20. Ascl-1, mammalian achaete-scute homologue 1; H&E, hematoxylin–eosin; OTP, orthopedia homeobox protein; SSTR-2A, somatostatin receptor 2A; TTF-1, thyroid transcription factor. Magnification, ×20.

and Ascl-1-IR ($n = 33$, 91.7%, $p = 0.0001$), and presence of SSTR-2A immunostaining ($n = 30$, 83.3%, $p < 0.0001$) (Figure 3F–J).

All the cases of oncocytic carcinoid were associated with the presence of conspicuous mitochondria in the cytoplasm, highlighted by strong and diffuse anti-mitochondria IHC marker expression.

Ki-67 labeling index was not significantly different in the two subgroups.

Among TCs with spindle cell morphology, there was a stricter association between predominant component (>50% of spindle cells) and the absence of SSTR-2A ($n = 18$, 94.7%, $p = 0.02$), the presence of Ascl-1-IR ($n = 13$, 68.4%, $p = 0.03$) and the presence of S100

protein in sustentacular cells ($n = 15$, 88.2%, $p = 0.009$) (supplementary material, Table S5).

DFS, OS, and CSS

Survival analysis revealed that patients with spindle cell morphology experienced longer CSS compared to those with ordinary morphology ($p = 0.04$; Figure 4C, Table 3). Groups with Ki-67 ≥3% and stage III–IV had a significantly worse OS (respectively, $p = 0.001$ and $p = 0.04$) and CSS ($p = 0.005$ and $p = 0.04$, respectively) than groups with stage I–II disease and Ki-67 <3% (Table 3, Figure 4A,B). Univariable analysis indicated that a 10-year increase in age ($p < 0.0001$), pN (1, 2, 3 versus 0, $p = 0.02$), and mitoses (1 versus 0, $p = 0.005$) were associated with poor OS; comparable findings were observed for CSS (Table 3).

Survival analysis demonstrated that the spindle cell variant had a better DFS compared to the ordinary histological variant ($p = 0.03$) (Table 3, Figure 5C). Kaplan–Meier analysis shows that patients with stage III–IV and Ki-67 ≥3% had worse DFS than patients with lower Ki-67 and stage I–II (respectively, log-rank $p = 0.012$ and $p < 0.0001$; Figure 5A,B). Univariable analysis (Table 3) identified significant clinicopathologic predictors of reduced DFS across the entire cohort of TCs: pT ($p = 0.045$), pN ($p = 0.007$), 10-year increase in age ($p < 0.0001$), and confirmation of anti-mitochondria expression ($p = 0.047$); while positivity for OTP ($p = 0.01$) was related to better prognosis.

Multivariable analysis and prognostic data

Table 4 shows information about the Cox multivariable proportional hazards regression analysis. After accounting for center variations, the multivariable analysis revealed that histological variants (spindle versus oncocytic, HR = 7.75, CI 1.57–38.12, $p = 0.012$; ordinary versus spindle, HR = 5.60, CI 1.34–23.46, $p = 0.018$), tumor stage (III versus I–II, HR = 3.14, CI 1.31–7.52, $p = 0.01$), age (10-year increase, HR = 1.71, CI 1.30–2.23, $p < 0.0001$), and Ki-67 (≥3 versus <3, HR = 5.74, CI 1.64–20.12, $p = 0.006$) were related to DFS, tumor stage, with Ki-67 the most significant negative clinicopathologic predictor for DFS, OS, and CSS (Table 4).

Discussion

TCs are rare well-differentiated LNENs that do not exhibit necrosis, have fewer than two mitoses per 2 mm^2, and show a staggering number of variant morphologies [1,2,9].

Oncocytic and spindle cell histological variants are acknowledged for their role in differential diagnosis, yet their clinical significance remains highly contested [10,11]. We investigated clinicopathological features of these two TC histological variants with the aim to shed light on their correlation with prognostic IHC markers and clinical outcome. We demonstrate that spindle cells, oncocytic, and nonordinary morphologic patterns are associated with different

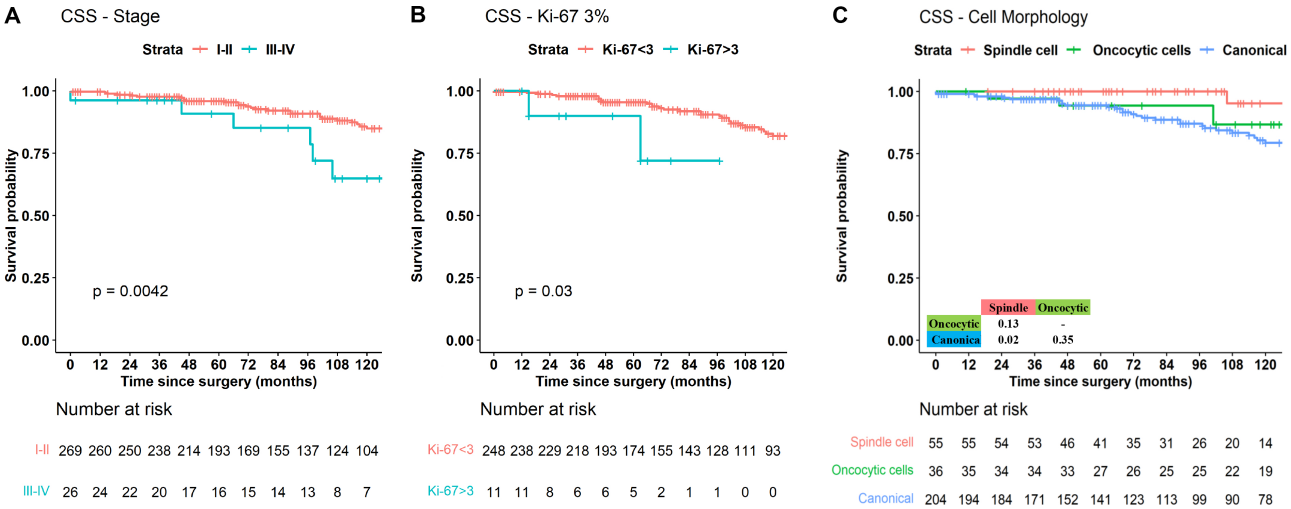

Figure 4. Cancer-specific survival (CSS) in typical carcinoids according to selected characteristics. (A) Stage; (B) Ki-67 3%; (C) cell morphology.

Table 3. Univariable* analysis of overall survival, cancer-specific survival, and disease-free survival of patients with typical carcinoids

Variable	OS, HR (95% CI)	*p*	CSS, HR (95% CI)	*p*	DFS†, HR (95% CI)	*p*
Sex (male versus female)	0.80 (0.46–1.39)	0.42	0.71 (0.35–1.45)	0.35	0.68 (0.35–1.29)	0.24
Years (10-year increase)	2.36 (1.79–3.11)	**<0.0001**	2.05 (1.49–2.81)	**<0.0001**	1.63 (1.26–2.10)	**<0.0001**
Smoking						
Former versus never smoker	0.83 (0.30–2.32)	0.72	0.77 (0.20–2.88)	0.69	0.31 (0.07–1.37)	0.12
Current versus never smoker	0.47 (0.17–1.32)	0.15	0.59 (0.16–2.18)	0.43	0.81 (0.31–2.11)	0.67
TCs and histological variants						
Oncocytic versus spindle	1.19 (0.36–3.96)	0.77	4.70 (0.52–42.2)	0.17	4.63 (0.98–22.37)	0.057
Canonical versus spindle	1.58 (0.61–4.06)	0.35	7.98 (1.08–58.82)	**0.04**	4.88 (0.96–22.37)	**0.03**
T (2–3–4 versus 1)	0.82 (0.44–1.53)	0.53	1.46 (0.73–2.93)	0.28	1.88 (1.02–3.48)	**0.045**
N (1/2/3 versus 0)	2.30 (1.17–4.50)	**0.02**	3.25 (1.55–6.82)	**0.002**	2.64 (1.30–5.37)	**0.007**
Stage (III–IV versus I–II)	3.82 (1.71–8.52)	**0.001**	3.42 (1.45–8.11)	**0.005**	4.77 (2.25–10.13)	**<0.0001**
Mitoses (1–2 versus 0)	2.19 (1.27–3.75)	**0.005**	2.23 (1.15–4.32)	**0.02**	1.61 (0.89–2.93)	0.12
Ki-67 (≥3 versus <3)	4.69 (1.07–20.57)	**0.04**	4.57 (1.04–20.06)	**0.04**	4.15 (1.24–13.91)	**0.02**
Vascular invasion (present versus absent)	0.69 (0.32–1.49)	0.35	0.70 (0.27–1.83)	0.46	0.67 (0.28–1.60)	0.36
Perineural invasion (present versus absent)	0.52 (0.13–2.14)	0.36	0.38 (0.05–2.79)	0.34	‡	‡
Intratumoral lymphocyte infiltrate (present versus absent)	0.72 (0.28–1.88)	0.50	0.69 (0.31–1.54)	0.36	0.82 (0.36–1.86)	0.64
Peritumoral lymphocyte infiltrate (present versus absent)	1.30 (0.66–2.57)	0.45	0.99 (0.40–2.43)	0.98	0.69 (0.29–1.65)	0.40
Location (peripheral versus central)	1.28 (0.73–2.25)	0.39	0.88 (0.43–1.79)	0.72	0.71 (0.36–1.38)	0.31
Microscopic infiltration						
Absent	1.00		1.00		1.00	
Positive STAS	1.33 (0.54–3.30)	0.54	1.18 (0.40–3.52)	0.76	0.97 (0.36–2.56)	0.94
Bronchus	0.62 (0.31–1.26)	0.19	0.60 (0.26–1.37)	0.22	0.51 (0.24–1.07)	0.08
Extra-lung	0.92 (0.30–2.85)	0.89	0.49 (0.10–2.39)	0.37	0.55 (0.15–2.04)	0.37
Morphological pattern (trabecular/nested/organoid versus insular/solid)	1.08 (0.55–2.14)	0.81	1.25 (0.56–2.77)	0.59	0.83 (0.43–1.62)	0.59
Residual tumor (R1/2 versus R0)	1.56 (0.47–5.15)	0.47	1.20 (0.28–5.10)	0.81	2.04 (0.71–5.86)	0.19
TTF-1 (present versus absent)	1.00 (0.31–3.25)	1.00	1.19 (0.36–3.95)	0.77	1.43 (0.56–3.67)	0.46
OTP (present versus absent)	0.60 (0.30–1.18)	0.14	0.57 (0.26–1.26)	0.17	0.42 (0.21–0.84)	**0.01**
SSTR-2A (present versus absent)	0.76 (0.41–1.38)	0.37	0.62 (0.31–1.27)	0.19	0.83 (0.44–1.59)	0.58
Ascl-1 (present versus absent)	1.48 (0.76–2.90)	0.25	1.02 (0.44–2.38)	0.96	0.82 (0.38–1.79)	0.62
Anti-mitochondria (present versus absent)	1.18 (0.62–2.23)	0.62	1.46 (0.69–3.09)	0.32	1.96 (1.01–3.80)	**0.047**
S100 (present versus absent)	1.05 (0.48–2.27)	0.91	1.01 (0.43–2.40)	0.98	0.89 (0.42–1.92)	0.77

Note: Significant *p* values are shown in bold font.

Ascl-1, mammalian achaete-scute homolog 1; CCS, cancer-specific survival; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; OS, overall survival; OTP, orthopedia homeobox protein; SSTR-2A, somatostatin receptor 2A; STAS, spread through air spaces; TCs, typical carcinoids; TTF-1, thyroid transcription factor 1.

*Adjusted for center.

†Evaluated on Stage I–II–II patients only.

‡Not estimable because there are no events.

clinicopathological features, IHC profile, and prognosis. Several studies have shown that TTF-1 immunoexpression in pulmonary TCs is most frequently encountered in the spindle cell type and peripherally located [12–15]. Papaxoinis *et al* classified a large number of resected TCs into three clusters, demonstrating that the TTF-1+/OTP+ subtype was most frequently peripherally localized and had spindle cells in variable proportion [12]. TTF-1 expression in TCs is not consistent: some studies have reported the absence of TTF-1 expression, while others document a variable range of lung-site marker expression (35–94%) [16–21]. We demonstrated TTF-1 presence in 10 of 36 TCs (27.8%), most of them with spindle cell morphology and peripheral site [14]. Our results also support the correlation between spindle cell morphology and peripheral location, the presence of TTF-1 and OTP immunoreactivity, and favorable outcome, in agreement with clinicopathological and IHC analysis of 13/80 predominant spindle cell component carcinoid tumors (≥50%) of the lung reported by Tsuta *et al* [13]. Moreover, our data confirm the prevalence, in peripheral spindle cell TCs, of S100 positive sustentacular cells (Figure 2) [22]. We described 36 out of 297 TCs (12%) exhibiting oncocytic features, although this histological variant is very rare [23]. The entire cohort of confirmed oncocytic carcinoids

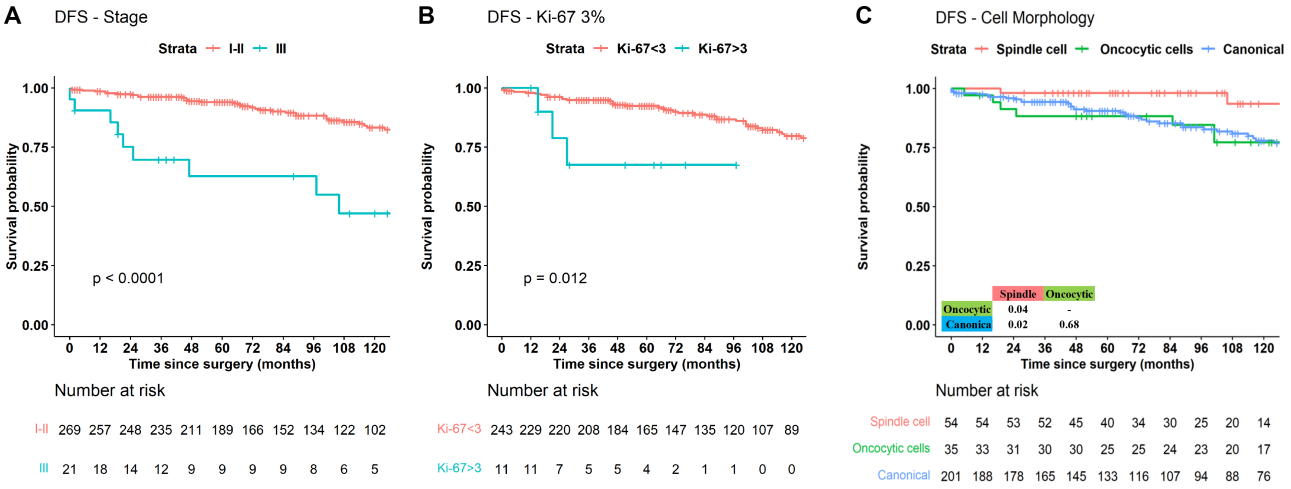

Figure 5. Disease-free survival (DFS) in typical carcinoids according to selected characteristics. (A) Stage; (B) Ki-67 3%; (C) cell morphology.

Table 4. Multivariable* models for overall survival, cancer-specific survival, and disease-free survival of patients with typical carcinoids

Variable	OS multivariable model, HR (95% CI)	*p*	CSS multivariable model, HR (95% CI)	*p*	DFS† multivariable model, HR (95% CI)	*p*
Years (10-year increase)	2.59 (1.91–3.52)	**<0.0001**	2.20 (1.57–3.08)	**<0.0001**	1.71 (1.30–2.23)	**<0.0001**
Cell morphology						
Oncocytic versus spindle	2.22 (0.65–7.57)	0.20	8.73 (0.96–79.57)	0.055	7.75 (1.57–38.12)	**0.012**
Canonical versus spindle	1.95 (0.76–5.04)	0.17	9.32 (1.26–68.69)	**0.03**	5.60 (1.34–23.46)	**0.018**
Stage (III–IV versus I–II)	3.55 (1.48–8.50)	**0.005**	3.00 (1.15–31.30)	**0.03**	3.14 (1.31–7.52)	**0.01**
Ki-67 (≥3 versus <3)	7.32 (1.60–33.51)	**0.01**	6.80 (1.48–31.30)	**0.01**	5.74 (1.64–20.12)	**0.006**

Note: Significant *p* values are shown in bold font.
*Adjusted for center.
†Evaluated on Stage I–II–-III patients only; HR considered for Stage III versus I–II.
CCS, cancer-specific survival; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; OS, overall survival.

was related to conspicuous mitochondria in the cytoplasm (Figure 4) [7,24,25], giving an eosinophilic cytoplasmic change, neurosecretory vesicles, lysosomal products, exocrine granules, cytoplasmic filaments, and sleek endoplasmic reticulum. We showed that in our TC series the vast majority of the oncocytic cells presented strong cytoplasmic granular immunoreactivity with anti-mitochondrial antibody. Tsuta *et al* demonstrated that a minor series of anti-mitochondrial positive oncocytic NETs (7/80, 9% of ONTs) were low-grade carcinoids and showed associations with earlier age at diagnosis, central location, and trabecular/organoid morphological pattern [23]. Regarding SSTR-2A receptors, we demonstrated, for the first time, that they are significantly more expressed in oncocytic TCs than in the two other variants (spindle cell and ordinary). SSTR-2A expression should be considered clinically when an option for SST-analogue therapy is needed. An additional recently reported finding is that some typical pulmonary oncocytic carcinoids show a deep fluorodeoxyglucose (FDG) uptake in FDG-PET/CT with one case expressing glucose transporter type 1 [26,27]. It must be remembered that FDG uptake in TCs is usually lower than more aggressive counterparts such as atypical carcinoids, large cell or small cell neuroendocrine carcinomas [28].

Our results suggest that pathologists should include neuroendocrine markers in the IHC panel when evaluating an oncocytic lesion.

Several prognostic factors have also been reported in literature like stage, Ki-67% and OTP. OTP serves as a distinct prognostic indicator for lung carcinoid tumors, as previously reported [29]. Swarts *et al* demonstrated that reduced levels of OTP are linked to worse disease outcomes and an higher risk of metastasis [30]. We independently confirmed and enhanced this evidence showing that OTP expression is associated with better OS, CSS, and DFS [31]. The present study on TCs confirms that OTP expression is associated with improved clinical outcome. In terms of

DFS, we demonstrated that spindle cell carcinoids presented a better survival than both oncocytic and ordinary variants; while the latter showed more tumor-associated deaths than others. Concluding, in the diagnostic context of TCs, morphological recognition by pathologists is strongly recommended to provide clinical outcome for these patients.

In summary, spindle cell, oncocytic, and ordinary TCs seem to exhibit distinct clinicopathological characteristics and prognostic impact. Ordinary TCs were associated with a higher number of tumor-related deaths compared to other histological variants. Spindle cell TCs showed a significantly longer DFS than both oncocytic and ordinary variants, as clearly defined by our survival analysis. These considerations further underline the need for multi-institutional initiatives to investigate and include a higher number of prognostic IHC markers in the diagnostic workup of TCs identifying patients with greater risk of progression and distant metastasis.

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Author contributions statement

Study concept and design – CC, GC, GS, PM, MM; methodology – GC, GS, VL, CP, GG, AM, MS; data analysis – GC, PM, CC, GS, MM; drafting of manuscript – GS, GC; critical revision of the manuscript for important intellectual content – CC, MM, AM, AF, SP, NP, AS, FG, LB, SG, LR, MRB, AB, LRol, UP; statistical analysis – GC, PM; study supervision – CC, MM.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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SUPPLEMENTARY MATERIAL ONLINE

Table S1. Antibody information

Table S2. Characteristics of patients with oncocytic variant typical carcinoids

Table S3. Characteristics of patients with spindle cell variant typical carcinoids

Table S4. Immunohistochemical markers of patients with oncocytic variant typical carcinoids

Table S5. Immunohistochemical markers of patients with spindle cell variant typical carcinoids