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ORIGINAL ARTICLE

Cytomorphologic and molecular characterization of spindle cell carcinoid tumors of the lung

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Abstract

Background: Spindle cell carcinoid tumor (SCCT) is a rare variant of lung carcinoid tumor consisting predominantly or exclusively of spindle cells. To the authors' knowledge, this is the first study to date investigating the molecular characteristics of SCCTs.

Methods: Eighty-five carcinoid tumors initially diagnosed by fine-needle aspiration over a period of 10 years were reviewed. The final diagnostic classification was based on resection specimens. Six SCCTs were identified and characterized based on cytomorphology, and immunohistochemical and molecular features.

Results: Most patients with SCCT were Caucasian (100.0%), women (83.3%), asymptomatic (66.7%), and nonsmokers (83.3%). The median age at diagnosis was 78.0 years (range, 58.2–80.3 years). A higher proportion of patients who had SCCT were diagnosed with distant metastasis. The smears were cellular and demonstrated clean backgrounds without necrosis or mitotic activity. SCCTs comprised of bipolar-to-elongated cells with finely granular chromatin, inconspicuous nucleoli, scant cytoplasm, and minimal atypia or pleomorphism. The tumor cells sometimes appeared *boomerang-shaped* and might mimic granulomas or blood vessels. SCCTs showed strong expression for pan-cytokeratin, synaptophysin, chromogranin, and CD56, with weak TTF-1 and a very low Ki-67 proliferation index. All SCCTs had low tumor mutational burden and were microsatellite-stable. One case showed multiple whole-gene losses in chromosome 11, whereas another harbored duplication in *ARID1A*. Two cases demonstrated gains in chromosomes 17, one of which also showed gains in chromosome 18. None had a single nucleotide mutation.

Conclusions: SCCT is a rare subset of lung carcinoid tumors. These tumors harbor unique cytologic, prognostic, and molecular features that may have significant diagnostic and clinical implications.

KEYWORDS
lung carcinoid, neuroendocrine tumor, next-generation sequencing, spindle cell carcinoid tumor, thoracic cytology, well differentiated neuroendocrine tumor

INTRODUCTION

Pulmonary neuroendocrine tumors, also known as carcinoid tumors, account for approximately 1%–2% of all lung malignancies.[1–4] The incidence rate of carcinoid tumors is slightly higher in Caucasians, and they usually occur in the fifth and sixth decades of life with a slight female preponderance.[4–6] There are two subtypes: typical carcinoids (TCs; carcinoid tumors with less than two mitotic figures per 2 mm^2 and lacking necrosis; 80%–90% of cases) and atypical carcinoids (ACs; carcinoid tumors with from two to 10 mitotic figures per 2 mm^2 and/or foci of necrosis, usually punctate; a minority of cases) recognized by the current International Agency for Research on Cancer and the World Health Organization classification of tumors group.[4,7,8]

Spindle cell carcinoid tumor (SCCT) is a rare morphologic variant of carcinoid tumor consisting predominantly (>50%) or exclusively of spindle cells. The first case of SCCT was reported by Felton et al. in 1953.[9] Since that original description, there have been variations in the definition of SCCT, and greater than 100 cases have been reported.[6,10] Most pulmonary SCCTs have been described in case reports or briefly named in references to neuroendocrine tumors.[5,6,10] In different series, it has been demonstrated that from 4.5% to 30.2% of all carcinoid tumors consist of SCCTs.[10] Although exceedingly rare, there have been some reports of SCCT in extrapulmonary locations, including the gastrointestinal tract and kidney.[11–13] In the lung, SCCTs are usually well demarcated, peripherally located, intraparenchymal lesions, ranging from 0.7 to 4.0 cm in size, with most of the neoplasms having a maximum dimension of ≥2.0 cm.[6,10] Because of their peripheral location, they are often clinically asymptomatic and most commonly encountered as an incidental finding on chest imaging.[6,10] SCCTs in most studies were described histologically as having an indistinct organoid pattern with sharply circumscribed borders and composed of elongated spindle cells with scant-to-moderate eosinophilic cytoplasm; the nuclei are uniform, centrally located, and round to oval with finely granular chromatin and inconspicuous nucleoli.[10] The differential diagnosis includes small cell carcinoma of the lung (SCC) and primary and metastatic mesenchymal tumors.[5,10,14,15] Immunohistochemical (IHC) analysis is useful in differential diagnosis; however, the few articles published on SCCTs emphasize its variable and unusual immunoprofile, which may represent a diagnostic pitfall.[10,14] To our knowledge, there has been no study on the molecular characteristics of SCCTs. The objective of this study was to analyze the molecular characteristics of SCCTs and compare their overall clinicopathologic characteristics with the features seen in TCs and ACs.

MATERIALS AND METHODS

Selection of cases

The carcinoid tumors diagnosed within a period of 10 years were retrieved from the University of Chicago Medical Center Department of Pathology archives. The cytologic smears, cell blocks, and surgical pathology slides were reviewed to confirm the diagnosis and subtype (i.e., TC, AC, SCCT), assess for any exclusion criteria (i.e., insufficient tissue), and document additional cytologic and histopathologic features (e.g., cellularity, architecture, mitotic figures, necrosis, atypia). The final diagnostic classification was based on resection specimens. Electronic medical records were reviewed to document smoking history, comorbidities, clinical presentation, management, and outcomes. This research was approved by the University of Chicago Institutional Review Board (IRB22-1221).

Immunohistochemical analysis

The following IHC analyses were performed on either the surgical pathology biopsy specimen or cytology cell block: pan-cytokeratin AE1/AE3 (clone AE1/AE3, Millipore; Sigma-Aldrich), CAM5.2 (clone CAM5.2; BD Biosciences), CK5/6 (clone D5; Dako), p40 (rabbit polyclonal; Abcam), synaptophysin (clone 27G12; Leica Biosystems), chromogranin (clone LK2H10; ThermoFisher Scientific), CD56 (clone MRQ-42; Cell Marque), TTF-1 (clone 867G3/1; Dako), napsin A (clone IP64; Leica Biosystems), and Ki-67 (clone MIB1; Dako). All IHC analyses were performed using validated protocols on either the Leica BOND-III platform (Leica Biosystems) or the BenchMark XT Ventana platform (Roche).

Molecular analysis

A representative Diff-Quik–stained cytology smear or a formalin-fixed, paraffin-embedded (FFPE) tumor block was selected for next-generation sequencing on the University of Chicago Medicine OncoPlus panel, a hybrid-capture panel targeting 1005 cancer-associated genes with 168 clinically reported genes, as previously described.[16] Seven cases of TCs and three cases of ACs were likewise sequenced. The variant review was performed by one of the authors (P.W.) and included filters based on population variant frequencies (The 1000 Genomes Project; https://www.internationalgenome.org; Accessed May 30, 2022), variant frequencies in cancer databases (COSMIC: Catalogue of Somatic Mutations in Cancer [https://cancer.sanger.ac.uk/cosmic; Accessed May 30, 2022] and cBioPortal [https://www.cbioportal.org; Accessed May 30, 2022]), and coding effects. Somatic variant calls were inspected using the Integrated Genomics Viewer (The Broad Institute, Massachusetts Institute of Technology). Copy number results were calculated using a combination of CNVkit[17] software and additional in-house intrarun normalization to eliminate run-specific artifacts by comparison with a pooled cohort of clinical controls.[18] Gene-level changes were called using the University of Chicago Medicine OncoPlus clinical interpretation criteria, as previously described.[16] This molecular test has been clinically validated on both Diff-Quik–stained cytology smears and formalin-fixed, paraffin-embedded tissue specimens.

Statistical analysis

The demographics, cytomorphology, immunohistochemistry, and molecular information were analyzed descriptively. The associations between molecular profiles and clinical, cytomorphologic, and IHC features were performed using the χ^2 test or the Fisher exact test, whichever was appropriate, for categorical variables. The Mann–Whitney *U* test or Kruskal–Wallis *H* test was used for continuous variables. Survival analysis was performed using Kaplan–Meier curves and a log-rank test. All hypothesis tests were two-sided, and statistical significance was set at $p < .05$. All statistical analysis was performed using IBM SPSS version 29 (IBM Corporation).

RESULTS

Clinical characteristics

Eighty-five carcinoid tumors were included in the study, consisting of 63 TCs (74.1%), 16 ACs (18.8%), and six SCCTs (7.1%). The final diagnoses were based on resection specimens. Most patients diagnosed with SCCT were Caucasian (100.0%), women (83.3%), and presented with incidental findings (66.7%; Table 1). The median age at diagnosis of patients with SCCT was 78.0 years (range, 58.2–80.3 years), which was nonsignificantly older than the median age of patients diagnosed with TCs (median, 66.8 years; range, 27.5–85.8 years) and ACs (median, 59.1 years; range, 33.0–87.8 years). The median serum chromogranin level was significantly higher in patients who had SCCT (502 ng/ml) compared with those who had ACs (69 ng/ml; $p = .027$) but were not significantly different from the serum chromogranin levels in patients who had TCs (137 ng/ml; $p = .569$). In contrast to patients who had TCs and ACs, most patients who had SCCTs were never-smokers (83.3%), which trended toward significance compared with the number of current/former smokers among patients who had TCs (54.0%; $p = .088$) and ACs (62.5%; $p = .056$). Significantly higher proportions of patients who had SCCTs were diagnosed with distant metastasis (16.7%) compared with those who had TCs (1.6%; $p = .017$) and ACs (0.0%; $p = .011$). The median overall survival of patients with SCCT was shorter at 5.2 months versus those with TC (14.2 months; log-rank $p = .468$) and AC (10.0 months; log-rank $p = .068$), but the differences did not reach statistical significance.

Cytopathologic features

On Romanowsky-stained (Diff-Quik) smears, SCCTs appeared as bipolar-to-elongated cells with scant cytoplasm and minimal atypia or pleomorphism (Figure 1). The tumor cells sometimes appear *boomerang-shaped* and may mimic granulomas (Figure 1A,B) or blood vessels (Figure 1A,C,D). A vague rosette formation was seen in all cases (Figure 1E), and focal areas of hyalinization were observed in two cases (Figure 1C,D). Plasmacytoid tumor cells were noted in one case (Figure 1D), and another case showed discohesive, cigar-shaped tumor cells (Figure 1F). The smears were cellular and demonstrated clean backgrounds without evidence of necrosis. No mitotic activity was noted in any SCCTs, similar to most TCs ($p = .324$; Table 2) and significantly different from ACs ($p < .002$; Table 2). The granular nuclear chromatin of tumor cells (salt-and-pepper chromatin) was better visualized with Papanicolaou staining (Figure 2). Nucleoli were inconspicuous. The scant cytoplasm and spindle-shaped tumor cells were also seen in this preparation, and the overall architecture mirrored those seen in Diff-Quik smears. Focal nuclear smearing was identified (Figure 2B). On hematoxylin-and-eosin–stained (H&E-stained) cell blocks, SCCTs appeared in small, organoid clusters (Figure 2D) or interanastomosing rosettes of spindled cells (Figure 2E) in a vaguely hyalinized-to-fibrillary background. Trabecular and pseudoglandular patterns were also identified with intervening blood vessels (Figure 2F).

Based solely on cytomorphologic analysis during rapid on-site evaluation, the initial diagnoses included spindle cell neoplasm ($n = 2$), epithelioid and spindle cell neoplasm ($n = 2$), and atypical spindle cells ($n = 2$).

Immunohistochemical analysis

IHC analysis was performed on cytology cell blocks ($n = 12$), surgical biopsy specimens ($n = 37$), or both surgical and cytology specimens ($n = 36$). When performed, all SCCTs showed strong cytoplasmic expression for synaptophysin (Figure 3C), chromogranin (Figure 3D), and CD56, with expression patterns similar to TCs. Pan-cytokeratin was likewise strongly positive in all cases (Figure 3B), whereas TTF-1 was weakly and focally positive in three cases (Figure 3E). Of note, one case (case 3) was negative for CAM5.2 but positive for AE1/AE3, suggesting the importance of using at least two pan-cytokeratin markers to confirm epithelial differentiation. The Ki-67 proliferation index ranged from 1% to 2%, showing weak focal staining (Figure 3F). Although no significant difference was noted, the median Ki-67 proliferation index of SCCTs was lower than that in TCs (median, 2%; range 0%–30%) and ACs (median, 10%; range, 0%–20%). All SCCTs were negative for p40. In general, the ICH features of SCCTs did not differ from those of TCs or ACs.

Based on combined cytomorphologic and IHC features, the cytologic diagnoses for the SCCTs included spindle cell neoplasm ($n = 1$); atypical cells consistent with spindle cell carcinoid ($n = 1$); epithelioid and spindle cell neoplasm, favor spindle cell carcinoid ($n = 1$); and spindle cell carcinoid ($n = 3$).

Molecular analysis

Molecular analysis was performed on all six SCCTs, seven TCs, and three ACs. DNA material was extracted from cytology Diff-Quik smears for six SCCTs, five TCs, and one AC and from formalin-fixed, paraffin-embedded surgical biopsy tissue specimens for two TCs and two ACs. The six SCCTs showed low tumor mutational

TABLE 1 Clinical characteristics of the patients diagnosed with lung carcinoid tumor.

Clinical features		No. (%)			
		Typical carcinoid, *n* = 63	Atypical carcinoid, *n* = 16	Spindle cell carcinoid, *n* = 6	*p* vs. TC/*p* vs. AC
Race	Caucasian	44 (69.8)	13 (81.3)	6 (100.0)	.379/.502
	African American	12 (19.0)	1 (6.3)	0 (0.0)	
	Mixed	3 (4.8)	0 (0.0)	0 (0.0)	
	Unknown	4 (6.3)	2 (12.5)	0 (0.0)	
Sex	Men	17 (27.0)	3 (18.8)	1 (16.7)	.596/.910
	Women	46 (73.0)	13 (81.2)	5 (83.3)	
Age at diagnosis: Median [range], years		66.8 [27.5–85.8]	59.1 [33.0–87.8]	78.0 [58.2–80.3]	.277/.088
Comorbidity	Cancer	39 (61.9)	11 (68.8)	2 (33.3)	.148/.052
	Noncancer	23 (36.5)	3 (18.8)	4 (66.7)	
	Unknown	1 (1.6)	2 (12.5)	0 (0.0)	
Smoking history	Never	29 (46.0)	6 (37.5)	5 (83.3)	.088/.056
	Current/former	34 (54.0)	10 (62.5)	1 (16.7)	
Clinical presentation	Incidental	40 (63.5)	9 (56.3)	4 (66.7)	.938/.658
	Respiratory symptoms	22 (34.9)	7 (43.7)	2 (33.3)	
	Unknown	1 (1.6)	0 (0.0)	0 (0.0)	
Clinical stage	I	7 (11.1)	2 (12.5)	0 (0.0)	.570/.029
	II	2 (3.2)	1 (6.3)	0 (0.0)	
	III	1 (1.6)	4 (25.0)	0 (0.0)	
	IV	9 (14.3)	0 (0.0)	2 (33.3)	
	Unknown	44 (69.8)	9 (56.3)	4 (66.7)	
Pathologic tumor classification	pT1	15 (23.8)	4 (25.0)	2 (33.3)	.679/.211
	pT2	8 (12.7)	6 (37.6)	0 (0.0)	
	pT3	2 (3.2)	2 (12.5)	0 (0.0)	
	pT4	1 (1.6)	0 (0.0)	0 (0.0)	
	Unknown	37 (58.7)	4 (25.0)	4 (66.7)	
Pathologic lymph node classification	pN0	21 (33.3)	3 (18.8)	2 (33.3)	.494/.151
	pN1	0 (0.0)	2 (12.5)	0 (0.0)	
	pN2	4 (6.3)	6 (37.5)	0 (0.0)	
	Unknown	38 (60.3)	5 (31.3)	4 (66.7)	
Pathologic metastasis classification	pM0	23 (36.5)	12 (75.0)	1 (16.7)	.017/.011
	pM1	1 (1.6)	0 (0.0)	1 (16.7)	
	Unknown	39 (61.9)	4 (25.0)	4 (66.7)	
Serum chromogranin: Median [range], ng/mL		137 [36–29,810]	69 [25–163]	502 [116–888]	.569/.027
Treatment	Observation	20 (31.7)	0 (0.0)	3 (50.0)	.516/.030
	Resection	26 (41.3)	7 (43.8)	1 (16.7)	
	Chemotherapy	3 (4.8)	1 (6.3)	1 (16.7)	
	Radiation	5 (7.9)	2 (12.5)	0 (0.0)	
	Combined/Other	9 (14.3)	6 (37.5)	1 (16.7)	
Recurrence	Yes	2 (3.2)	1 (6.3)	0 (0.0)	.670/.567
	No	56 (88.9)	14 (87.5)	4 (66.7)	

TABLE 1 (Continued)

Clinical features		No. (%) Typical carcinoid, *n* = 63	Atypical carcinoid, *n* = 16	Spindle cell carcinoid, *n* = 6	*p* vs. TC/*p* vs. AC
	Unknown	5 (7.9)	1 (6.3)	2 (33.3)	
Status at follow-up	Alive, no evidence of disease	10 (15.9)	3 (18.8)	1 (16.7)	.133/.640
	Alive with disease	39 (61.9)	11 (68.8)	3 (50.0)	
	Died of disease	8 (12.7)	0 (0.0)	0 (0.0)	
	Died of other cause	1 (1.6)	1 (6.3)	0 (0.0)	
	Lost to follow-up	5 (7.9)	1 (6.3)	2 (33.3)	
Overall survival: Median [range], months		14.2 [0.0–84.3]	10.0 [0.0–74.8]	5.2 [0.3–33.1]	.468/.068

Abbreviations: AC, atypical carcinoid; TC, typical carcinoid.

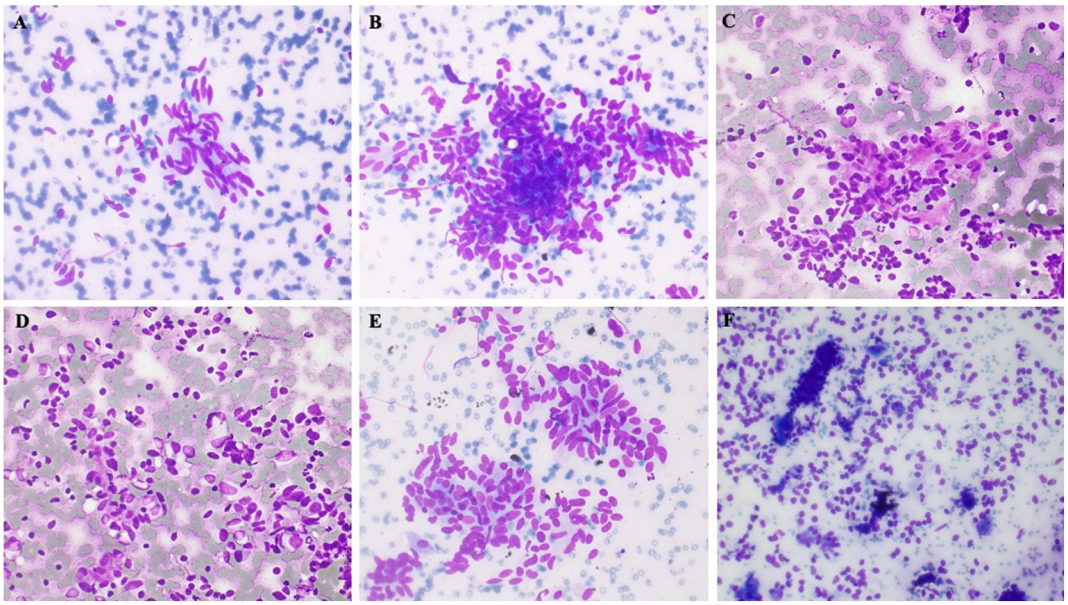

FIGURE 1 Cytomorphology of the six spindle cell carcinoid tumors in Diff-Quik smears. Spindle cell carcinoid tumors appeared as bipolar-to-elongated cells with scant cytoplasm and minimal atypia or pleomorphism. The tumor cells sometimes appeared *boomerang shaped* and might mimic (A,B) granulomas or (A,C,D) blood vessels. (E) Vague rosette formation was seen in all cases, and (C,D) focal areas of hyalinization were observed in two cases. (D) Plasmacytoid tumor cells were noted in one case, and (F) another case showed discohesive, cigar-shaped tumor cells. The smears were cellular and demonstrated clean backgrounds. No mitotic activity or necrosis was noted.

burden (TMB), with a median TMB of 2.8 mutations per Mb (range, 0.4–3.3 mutations per Mb), and all were microsatellite stable (Figure 4A). Cases 1 and 2 did not show any pathogenic alterations or chromosomal number variations. Case 3 showed multiple whole-gene losses involving *KMT2A*, *CHEK2*, *ATM*, *CHEK1*, *CBL*, *BIRC3*, *MRE11*, and *FAT3*. Case 5 harbored duplication in *ARID1A*. Cases 4 (Figure 4B) and 6 (Figure 4C) did not exhibit any pathogenic mutations but demonstrated gains in chromosome 17 (cases 4 and 6) and 18 (case 6).

Seven TCs and three ACs were sequenced as controls. All cases showed low TMB and microsatellite stable status (Figure 4A). The TCs showed pathogenic alterations in *ARID1A* (*n* = 2), *KMT2A* (*n* = 2), *CCND2* (*n* = 1), *CCND1* (*n* = 1), *HRAS* (*n* = 1), *KRAS* (*n* = 1), *ALK* (*n* = 1), *NOTCH1* (*n* = 1), *SDHD* (*n* = 1), and *STAG2* (*n* = 1). Among the ACs, two tumors showed *RB1* alterations, one of which had an additional *ARID1A* mutation, and the third tumor harbored deletion and frameshift mutation of *CHEK2*. None of the TCs and ACs showed *TP53* alterations.

DISCUSSION

This is the first study to date reporting the molecular characteristics of SCCTs. In contrast to TCs and ACs, SCCTs did not show single nucleotide variations, and most had whole-gene and chromosomal

TABLE 2 Immunopathologic features of carcinoid tumors.

Pathologic features		No. (%) Typical carcinoid, *n* = 63	Atypical carcinoid, *n* = 16	Spindle cell carcinoid, *n* = 6	*p* vs. TC/*p* vs. AC
Mitosis per 10 HPF: Median [range]		0 [0–9]	3 [0–17]	0 [0–0]	.324/.002
Ki-67 proliferation index: Median [range], %		2 [0–30]	10 [0–20]	2 [1–2]	1.000/.746
Synaptophysin	Positive	60 (95.2)	13 (81.3)	5 (83.3)	1.000/1.000
	Negative	0 (0.0)	1 (6.3)	0 (0.0)	
	NP	3 (4.8)	2 (12.5)	1 (16.7)	
Chromogranin	Positive	57 (90.5)	12 (75.0)	4 (66.7)	1.000/.567
	Negative	0 (0.0)	1 (6.3)	0 (0.0)	
	NP	6 (9.5)	3 (18.7)	2 (33.3)	
CD56	Positive	14 (22.2)	5 (31.3)	6 (100.0)	NA/NA
	Negative	0 (0.0)	0 (0.0)	0 (0.0)	
	NP	49 (77.8)	11 (68.7)	0 (0.0)	
TTF-1	Positive	13 (20.6)	7 (43.7)	3 (50.0)	.128/.521
	Negative	11 (17.5)	1 (6.3)	3 (50.0)	
	NP	39 (61.9)	8 (50.0)	0 (0.0)	
Napsin A	Positive	1 (1.6)	0 (0.0)	0 (0.0)	NA/NA
	Negative	1 (1.6)	0 (0.0)	0 (0.0)	
	NP	61 (96.8)	16 (100.0)	6 (100.0)	
Pancytokeratin	Positive	25 (39.7)	4 (25.0)	6 (100.0)	.730/.408
	Negative	1 (1.6)	1 (6.3)	0 (0.0)	
	NP	37 (58.7)	11 (68.7)	0 (0.0)	

Abbreviations: AC, atypical carcinoid; HPF, high-power fields; NA, not applicable; NP, not performed; TC, typical carcinoid.

copy number alterations. Distinct clinicopathologic characteristics were likewise observed in this study. Patients who had SCCTs were never-smokers, diagnosed at an older age, and had a higher rate of distant metastasis. On smears, the SCCTs did not demonstrate any necrosis or mitotic activity, similar to TCs. The IHC findings in SCCTs were similar to those in TCs and ACs with the exception of one case that was negative for CAM5.2. Based on combined morphologic and IHC findings, SCCTs had more similarity with TCs than with ACs.

The clinicopathologic characteristics of SCCTs were previously reported by Tsuta et al., who investigated 13 SCCTs of 80 consecutively diagnosed carcinoid cases (16.3%).[10] Only four tumors (30.7%) in their cohort were positive for pan-cytokeratin.[10] Although we observed a higher positivity rate (83.3%) for pan-cytokeratin, the negative staining observed in one of our six cases suggests that loss of broad-spectrum keratin is not uncommon in SCCTs and must be recognized to avoid misdiagnosis as a mesenchymal tumor, such as monophasic synovial sarcoma. Transducin-like enhancer of split (TLE1) has recently emerged as a biomarker with expression in SCCT because it plays a role in tumorigenesis of the lung among other organs and can also lead to the misdiagnosis of SCCT as synovial sarcoma.[14,19] The latter is usually negative for keratins, TTF-1, and neuroendocrine markers, which may be helpful in the distinction.[14] The use of the fusion protein SS18-SSX antibodies can be an adjunctive test to help differentiate between the two in challenging cases because this gene fusion is specific to synovial sarcoma.[14,19,20] Of note is that TLE1 has a direct role in lung cancer because of its interaction through E-cadherin, which is a prime regulator of epithelial-mesenchymal transition in cancer cells of the lung. Malignant bronchial epithelial cells have an increase in TLE1 expression with downstream effects that lead to the suppression of E-cadherin through transcription factor zinc finger E-box binding homeobox 1 (ZEB1).[19]

The vast majority of reported SCCT cases were of the TC subtype and/or had a low Ki-67 index.[5,21] A high Ki-67 index is a known poor prognostic factor in pulmonary carcinoid tumors.[5,22] In the study by Tsuta and colleagues, the mean Ki-67 index was 1.8% and within the range for TCs.[10,22] In the current study, the Ki-67 proliferation index showed weak focal staining with indices from 1% to 2%. Although no significant difference was noted, the median Ki-67 proliferation index of SCCTs was lower than the that of TCs (median, 2%; range, 0%–30%) and ACs (median, 10%; range, 0%–20%).

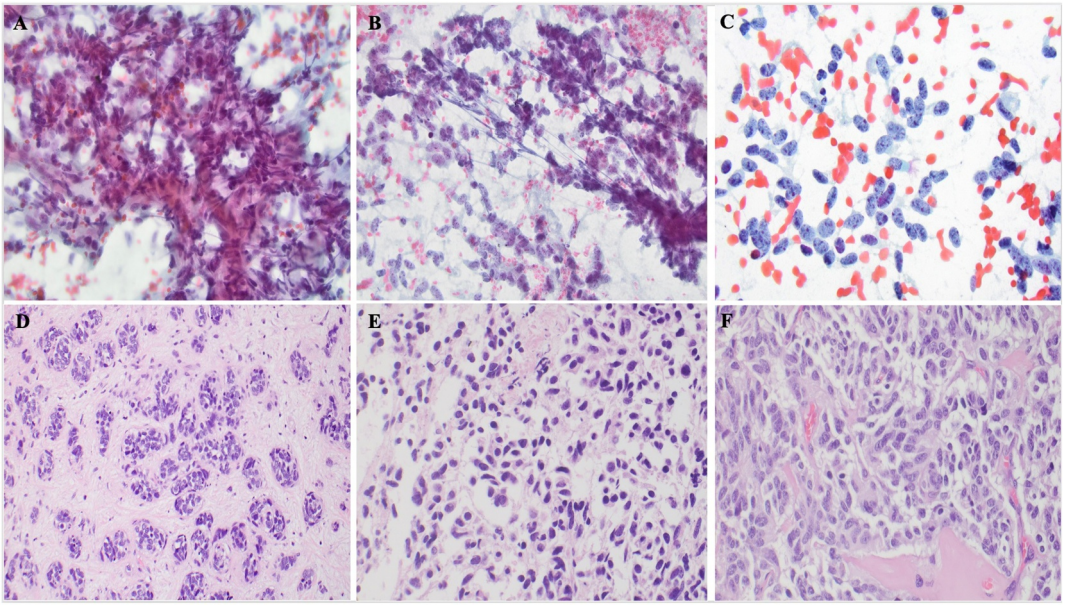

FIGURE 2 Cytomorphology of spindle cell carcinoid tumors (SCCTs) in Papanicolaou smears and cell blocks. (A–C) These smears highlight the finely granular nuclear chromatin of tumor cells (salt-and-pepper chromatin). Nucleoli are inconspicuous. The scant cytoplasm and spindle-shaped tumor cells are likewise seen in this preparation, and the overall architecture mirrors that seen in Diff-Quik smears. (B) Focal nuclear smearing is identified. On H&E-stained cell blocks, spindle cell carcinoid tumors appear (D) in small, organoid clusters or (E) as interanastomosing rosettes of spindled cells in a vaguely hyalinized-to-fibrillary background. (F) Trabecular and pseudoglandular patterns are also identified with intervening blood vessels.

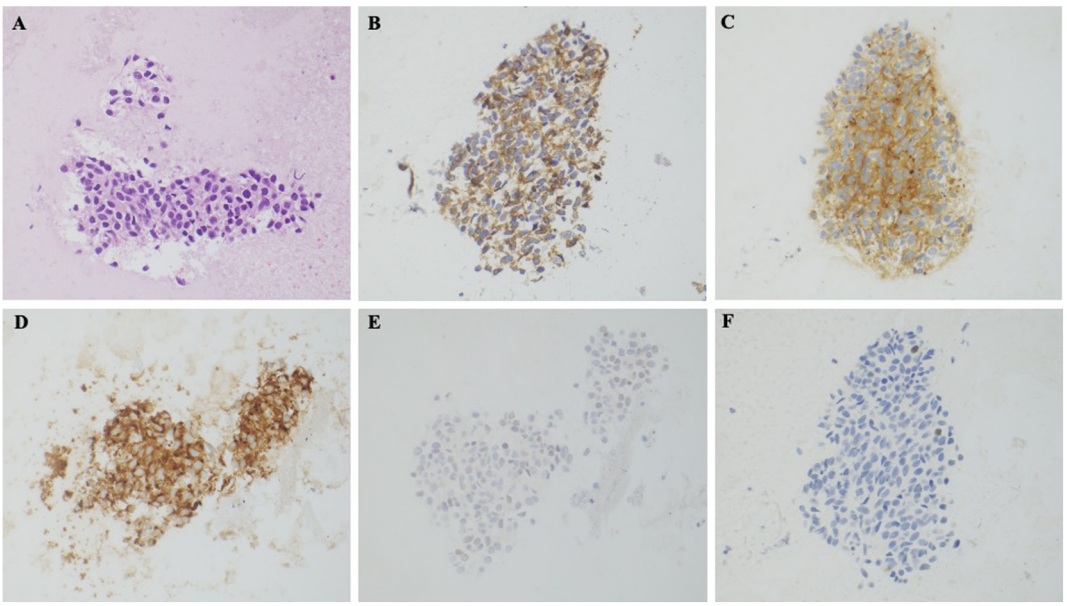

FIGURE 3 Immunohistochemical analysis of spindle cell carcinoid tumors. When performed, all spindle cell carcinoid tumors showed strong cytoplasmic expression for (B) pan-cytokeratin, (C) synaptophysin, (D) chromogranin, and CD56, with expression patterns similar to typical carcinoids. (E) TTF-1 was weakly and focally positive in three cases, whereas (F) the Ki-67 proliferation index ranged from 1% to 2%, showing weak focal staining. (A) Demonstrates a representative cell block of a spindle cell carcinoid tumor.

Similar to the patients with SCCT in the study of Tsuta et al., the current cohort was mostly clinically asymptomatic.[10] However, although some studies have observed favorable outcomes in patients with SCCT,[5,10] two patients in the current study were diagnosed with metastatic disease, and their overall survival seemed shorter (5.2 months) compared with the patients who had TCs (15.1 months) and ACs (8.1 months). However, these differences did not reach statistical significance, and the interpretation may be limited because of the short follow-up period and because two patients were lost to follow-up. The reports on the clinical behavior of SCCTs range from

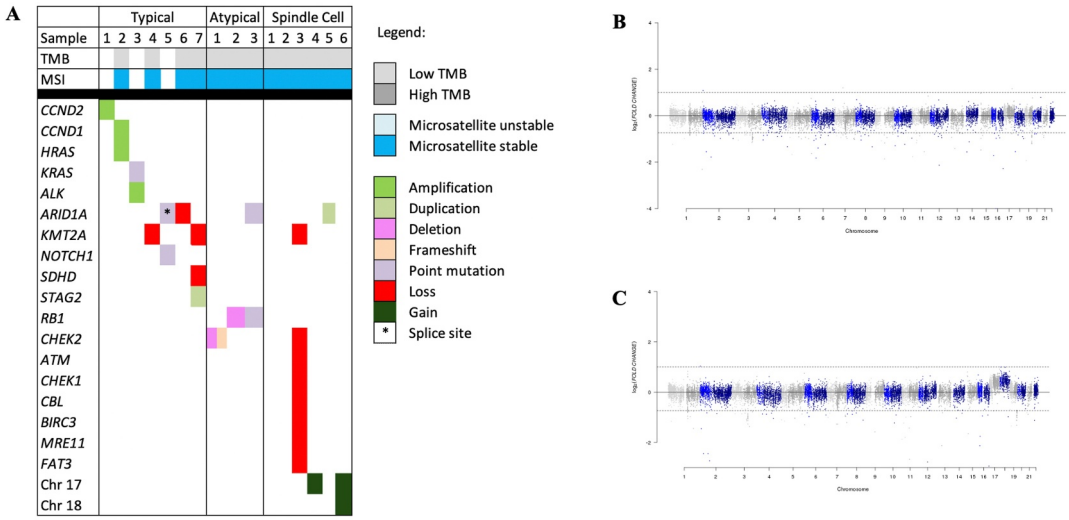

FIGURE 4 Molecular plot and chromosomal analysis of the carcinoid tumors. (A) The six spindle cell carcinoid tumors showed low TMB and were microsatellite stable. Cases 1 and 2 did not show any pathogenic alterations or chromosomal number variations. Case 3 showed multiple whole-gene loss involving *KMT2A*, *CHEK2*, *ATM*, *CHEK1*, *CBL*, *BIRC3*, *MRE11*, and *FAT3*. Case 5 harbored duplication in *ARID1A*. (B) Case 4 and (C) case 6 demonstrated gains in chromosome 17 (cases 4 and 6) and 18 (case 6). All typical and atypical carcinoids likewise showed low tumor mutational burden and microsatellite stable status. The typical carcinoids showed pathogenic alterations in *ARID1A* ($n = 2$), *KMT2A* ($n = 2$), *CCND2* ($n = 1$), *CCND1* ($n = 1$), *HRAS* ($n = 1$), *KRAS* ($n = 1$), *ALK* ($n = 1$), *NOTCH1* ($n = 1$), *SDHD* ($n = 1$), and *STAG2* ($n = 1$). The atypical carcinoids showed *RB1* alterations ($n = 2$), an *ARID1A* point mutation ($n = 1$), and deletion and frameshift mutation of *CHEK2* ($n = 1$). MSI indicates microsatellite instability; TMB, tumor mutational burden.

extremely indolent to more aggressive than non-SCCTs, with regional lymph node metastases reported in approximately 20% in some series.[6,10,12] The average mitotic activity and Ki-67 proliferation index of SCCTs in this cohort were lower than both TCs and ACs. In a study by Centonze et al., those authors reported that a Ki-67 proliferation index cutoff of 3% can predict disease-free survival in patients with lung carcinoid tumors.[23] The discordance between the seemingly low-grade cytomorphologic appearance of SCCTs and the unfavorable clinical presentation warrants further investigation to be performed in a larger, multi-institutional patient cohort with longer clinical follow-up.

Research genomic studies indicate that pulmonary carcinoid tumors have a low mutation rate. Significantly mutated genes include *MEN1*, *EIF1AX*, and *ARID1A*.[24–26] Mutations in chromatin-remodeling genes, such as those involved in histone methylation and acetylation, as well as members of the SWI/SNF complex, are present in 50% of cases.[1] Overall, *MEN1* is the most frequently mutated gene, with somatic mutations in 11%–22% of cases.[1,24,25] *ARID1A* mutations were observed in the current cohort of TCs, whereas duplication of *ARID1A* was seen in one SCCT case. *ARID1A* encodes for a factor that promotes the formation of SWI/SNF-mediated chromatin remodeling and functions as a tumor suppressor.[1,26] Duplication of this gene may have an effect similar to that of an *ARID1A* mutation because this genetic alteration is a known tumorigenic pathway in TCs and ACs.[24–26]

The SCCTs in this cohort were genomically stable without any single nucleotide variation. However, one case showed whole-gene loss involving *KMT2A*, *CHEK2*, *ATM*, *CHEK1*, *CBL*, *BIRC3*, *MRE11*, and *FAT3*. Except for *CHEK2*, all of these genes are located in the long arm of chromosome 11 (www.omim.org; Accessed June 30, 2023). The loss of heterozygosity on chromosome 11q is a known genomic alteration observed in sporadic (nonsyndromic) lung and gastric carcinoids, with loss of certain loci seen in association with atypical features and a poor clinical outcome.[27–29] Notably, chromosome 11 is also the location of *MEN1*, the loss of which was observed more commonly in ACs compared with TCs.[30] *CHEK2* loss is rare in lung carcinoids, seen in only 0.03% of lung tumors and 0.1% in other cancers.[31] *CHEK2* is a tumor suppressor gene that encodes the protein CHEK2, a serine-threonine kinase involved in DNA repair and cell cycle checkpoint regulator.[32] Alterations in this gene are associated with genetic predisposition to sarcomas and breast cancer.[31–33] In addition to one SCCT with *CHEK2* loss, one AC in this current cohort showed deletion and frameshift mutation of *CHEK2*. The tumorigenic role of this gene in lung carcinoids may warrant further investigation.

Unlike in SCC and large cell neuroendocrine carcinoma, mutations in the *TP53* and *RB1* tumor suppressor genes are extremely rare in pulmonary carcinoids, and the minority of ACs that show *TP53* mutations lack the smoking-related G>T and C>A transversions typically found in SCC and large cell neuroendocrine carcinoma.[25,34,35] In the current study, no tumor had *TP53* alteration, whereas two ACs showed *RB1* alterations. *RB1* alterations are seen in approximately 5% of lung cancers and are identified frequently in lung adenocarcinoma, SCC, and large cell neuroendocrine carcinoma and rarely in ACs.[25,30] The presence of *TP53* and *RB1* alterations in ACs had been associated with poor clinical outcomes.[25,36,37] The

term *supracarcinoid* has been suggested to describe a unique subset of carcinoid tumors with well differentiated morphology but harbor molecular alterations that define neuroendocrine carcinomas, specifically loss of *RB1* and *TP53* mutations.[36,37] These tumors tend to behave aggressively and were proposed to be the early molecular transition of carcinoid tumors to neuroendocrine carcinoma.[36,37] The two *RB1*-mutated ACs in this current cohort had an increased Ki-67 proliferation index of 10% and 20%. These patients were managed with combined resection (lobectomies) and chemotherapy. Although neither patient had a documented recurrence, they still had persistent disease based on the latest available clinical notes, which were accessed after relatively short follow-up periods of 4.4 and 20.6 months. The limited follow-up period precludes any definitive prognostic conclusions.

One limitation of this study is the small number of SCCT cases compared with TCs and ACs. Although the incidence rate of SCCTs in the current cohort was at the lower end of the frequency range from prior investigations, the diagnostically and clinically significant findings in this cohort serve as relevant pilot material for future, larger multi-institutional studies that will allow for stronger statistical comparisons.

In summary, this study highlights the unique clinical, cytomorphologic, and molecular characteristics of SCCTs compared with other types of lung carcinoid tumors. SCCTs appear morphologically low-grade and genomically stable but may have potentially poor clinical outcomes.

AUTHOR CONTRIBUTIONS

Rachelle P. Mendoza: Methodology, data curation, investigation, formal analysis, visualization, writing–original draft, and writing-reviewing and editing. **Emily Symes**: Data curation and writing-reviewing and editing. **Peng Wang**: Data curation, formal analysis, and writing–reviewing and editing. **Cole Miller**: Writing–original draft. **Stephanie C. Thompson**: Writing–original draft. **Tatjana Antic**: Data curation, supervision, and writing–reviewing and editing. **Anna Biernacka**: Conceptualization, data curation, supervision, writing–original draft, and writing–reviewing and editing.

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CONFLICT OF INTEREST STATEMENT

The authors disclosed no conflicts of interest.

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