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Discussion Articles

Savari O, et al. Non-small Cell Lung carcinoma with Diffuse Coexpression of TTF1 and p40: Clinicopathological and Genomic Features of 14 Rare Biphenotypic Tumors. *Histopathology*. 2023;82(2):242-253.

Purpose: Non-small cell lung carcinomas (NSCLC) with diffuse coexpression of TTF1 and p40 have been described in rare case reports. This study collected 14 such cases and investigated their clinicopathological and genomic characteristics.

Methods: The reported TTF1 expression in lung squamous cell carcinoma (SqCC) is usually seen in the less specific TTF1 clones (SPT24 and SP141). When the more specific TTF1 clone 8G7G3/1 is used, the expression of TTF1 in lung SqCC is limited to rare cases with only focal and/or weak staining. Similarly, the expression of p40 in lung adenocarcinoma is rare and limited to scattered neoplastic cells. So far, only 5 cases with diffuse coexpression of TTF1 and p40 are reported. 14 cases with diffuse (i.e., 50-100% of tumor cells) coexpression of TTF1 and p40 are collected among 3 institutions (Memorial Sloan Kettering Cancer Center, Columbia University Medical Center, and University of North Carolina). Targeted NGS using MSK-IMPACT (up to 468 cancer genes for 7 cases) and SLI-Mamp platform (9 cancer genes for 1 case) was performed for 8 cases. The NGS results of the 8 cases are compared to 9181 lung adenocarcinomas and 1066 lung SqCCs from the MSK-IMPACT prospective clinical sequencing cohort.

Results:

- Table 2. *Patient characteristics and radiological features*

14 cases	Age	Gender	Smoking Hx	Pack yrs	Pulmonary Location	Tumor size (cm)	PET SUV	Stage	Specimen type
Summary	Median 78	M 50% (7/14)	Smoker 89% (8/9)	Median 30	Peripheral 80% (8/10)	2.9	Median 8.6	Stage III/IV 50% (5/10)	Biopsy/FNA 64% (9/14)

- Table 3. *Histopathological and immunohistochemical features*

14 cases	Histology			IHCs and mucicarmine				
	Basaloid features	Keratinization	Necrosis	P40	TTF1	CK5/6	Napsin A	Mucicarmine
Summary	43%	14% (focal, <1% of tumor)	50%	Median reactivity: 90%	Median reactivity: 80%	Labeling in 92% of cases	Labeling in 39% of cases	Labeling in 25% of cases

Neuroendocrine markers are all negative.

- *NGS results* (Figure 4):
  - recurrent mutated genes include *TP53* (7/7), *CDKN2A* (3/7), *KRAS* G12C (2/7)
  - *EGFR* exon 20 insertion is seen in 1 case
  - recurrent copy number alterations: *FGFR1* amplifications (5/7), *MYC* amplification (3/7), *AKT* amplification (2/7), *NKX2.1* (encoding TTF1) amplification (2/7)
  - Comparing to lung adenocarcinoma (n > 9k) and lung SqCC (n > 1k), the rate of FGFR1 amplifications in this cohort is significantly higher than that in lung adenocarcinomas ($P < 0.0001$) or lung SqCC ($P < 0.001$).
- *Clinical follow up* (Table 4): follow up available for 8 patients for 1.3 to 55 months- 5 with metastatic disease, 4 died, 3 alive with disease, 1 with no evidence of disease after resection

Take-home message: NSCLC with diffuse coexpression of TTF1 and p40 morphologically resemble non-keratinizing SqCC with a significant proportion with basaloid features. By IHC and mucicarmine stain, dual (squamous + glandular) differentiation is demonstrated, which is in line with previous EM findings of bilineage differentiation in these tumors at a single cell level (Pelosi G, et al. JTO Clin. Res. Rep. 2021;2;100222). While the overall genomic features are more similar to SqCCs, they also show typical adenocarcinoma-type gene alterations These tumors should undergo molecular testing, given that 3 of 8 showed targetable gene mutations. The unique high rate of *FGFR1* amplification may offer an opportunity for targeted therapy. These tumors have an aggressive clinical course. These tumors are proposed to arise from TTF1/p40 dual positive bronchiolar basal cells. The proposed name is “NSCLC with biphenotypic differentiation”.

Centonze G, et al. Lung Carcinoid Tumors: Histology and Ki-67, The Eternal Rivalry. *Histopathology*. 2023;82(2):324-339.

Purpose: The role of Ki67 in pulmonary neuroendocrine neoplasm (NEN) is not well established. While Ki67 labeling index plays a key role for both diagnosis, grading, and prognosis of NEN in gastroentero-pancreatic (GEP) system, the current WHO classification for thoracic tumors does not recommend Ki67 as a mandatory assessment for pulmonary NENs. This study is to evaluate the role of Ki67 along with other immunohistochemical markers (including TTF1, CD44, Orthopedia Homeobox <OTP>, SSTR-2A, Ascl1, and p53) in predicting disease aggressiveness for both typical carcinoid tumors (TC) and atypical carcinoid tumors (AC).

Methods: 355 cases of carcinoid tumors, including 297 TCs and 58 ACs, are included in this study from 2 Italian oncology centers between 1988 and 2018. Ki67 labeling index is counted as positive cells% in 500-2000 tumor cells in hot spots.

Results:

- Clinicopathologic features and treatment

Histology		Sex		Age	Stage				Pre and/or postoperative Treatment (n=217)		
TC	AC	F	M	Median: 60 yrs	I	II	III	IV	Somatostatin antagonist	Chemo/radiation	No treatment
297 (84%)	58 (17%)	199 (62%)	118 (38%)		264 (74%)	48 (14%)	33 (9%)	10 (3%)	14 (7%)	10 (5%)	193 (88%)

- Ki-67 labeling index (n=317): 0-26% (median:1.1%); 3 cases had >20% (analogous to G3 GEP-NET)

- Figure 2: Using ROC curve analysis, 3% is the best cut off value for Ki-67 to predict disease-free survival (DFS)

- Using 3% cut off, tumors with Ki-67 >=3% are associated with AC histology ($p<0.0001$), stage III-IV ($p=0.004$), smoking hx ($p=0.001$), presence of necrosis ($p<0.0001$), vascular invasion ($p=0.01$), peritumoral lymphocyte infiltrate ($p=0.02$), presence of STAS ($p<0.0001$) and solid growth pattern ($p<0.0001$) (Table 1).

- Figure 3, Table 3, and Figure 4: Overall survival (OS), cancer-specific survival (CSS) and disease-free survival (DFS)

  - 58 patients (16%) showed tumor-associated death out of 78 (22%) total deaths

  - In univariate analysis, Ki-67>=3%, AC histology, mitosis, age (10-years increase), lymph node involvement (N1/2/3), higher stage (III-IV), or absence of expression of OTP correlates with significantly worse CSS ($P<0.0001$). (Figure 3 and table 3)

  - Similarly, Ki-67>=3%, AC histology, lymph node involvement (N1/2/3), or absence of expression of OTP correlates with significantly worse DFS ($P<0.0001$) (Figure 4)

- Using multivariable cox proportional regression analysis, AC histology (HR 3.68; $p<0.0001$) and high Ki-67 (HR 3.35, $p=0.0004$) were the strangest predictors for CSS. Similar results were seen for OS and DFS (table 4).

- When Ki-67, histology, or both are added to the multivariable models in terms of OS, CSS, and DFS, Ki-67 or histology alone adds significant prognostic information in a multivariable model for both OS and CSS. But adding both variables does not provide further prognostic information for OS and CSS. Interestingly, for DFS, adding Ki-67 to a model containing histology showed improved significance of the prediction model, whereas adding histology to Ki-67 did not (table 5).

Take-home message: Ki-67 at a 3% cut-off is a good prognostic marker with strong association with post-operative recurrence for pulmonary carcinoid tumors. More studies are needed to validate the proposed 3% Ki-67 cut off in separating TCs from ACs. More studies are needed to for highly proliferative carcinoids with mitosis > 10 / $2mm^2$ and/or Ki67 > 20% to validate that a category of grade 3 NET as proposed in GEP-NEN system does exist in the lung as a distinct prognostic group different from TCs, ACs, LCNECs, and small cell carcinomas.

Sa-Ngiamwibool P, et al. Usefulness of NF2 Hemizygous Loss Detected by Fluorescence In Situ Hybridization in Diagnosing Pleural Mesothelioma in Tissue and Cytology Material: A multi-Institutional Study. Lung Cancer. 2023;175:27-35.

Purpose: *NF2* (ch22q12) genetic alteration is specific for malignant mesothelial proliferation and has been observed in up to 68% pleural mesotheliomas (Chapel DB et al. Mod Pathol. 2022;35(10):1383-1397). This study is to investigate the diagnostic utility of *NF2* hemizygous loss (HL) by FISH for diagnosing malignant pleural mesotheliomas in combination with other diagnostic tests including BAP1 IHC and *CDKN2A* FISH. In addition, the *NF2* FISH result is further analyzed regarding its correlation with overall survival, histologic subtypes, and histological grading of epithelioid mesothelioma.

Methods: The study cohort includes 106 surgical and 107 cytology specimens of pleural mesotheliomas from 6 Japanese hospitals. The control group includes 37 surgical and 31 cytology specimens of benign mesothelial proliferation

Results:

- *NF2* hemizygous loss by FISH (Table 1): no *NF2* hemizygous loss in surgical (n=37) and cytology (n=31) cases for benign mesothelial proliferation

	NF2 FISH, surgical cases (n=106)		*NF2* FISH, cytology cases (n=107)	
	Hemizygous loss (n=58; 54.7%) 54 monosomy, 4 heterozygous deletion	NML (n=48)	Hemizygous loss (n=53; 49.5%) 47 monosomy, 6 heterozygous deletion	NML (n=44)
Epithelioid	47 (81%)	34 (71%)	51 (96%)	53 (98%)
Biphasic	8 (14%)	6 (13%)	2 (4%)	1 (2%)
Sarcomatoid	3 (5%)	8 (16%)		

- Grading of epithelioid mesothelioma does not correlate with *NF2* hemizygous loss, *CDKN2A* homozygous deletion, or BAP1 loss (Table 2).

- Sensitivity and specificity of various assays in detecting pleural mesotheliomas (Table 3)

	BAP1 IHC	*CDKN2A* HD FISH	*NF2* HL FISH	BAP1 + *CDKN2A*	BAP1 + *NF2*	*CDKN2A* + *NF2*	BAP1 + *CDKN2A* + *NF2*
Sensitivity	Surgical: 65.4% Cytology: 61.5%	Surgical: 75.5% Cytology: 63.6%	Surgical: 54.7% Cytology: 49.5%	Surgical: 94.2% Cytology: 88.5%	Surgical: 84.6% Cytology: 76.6%	Surgical: 91.5% Cytology: 82.2%	Surgical: 98.1% Cytology: 94.4%
Specificity	100%	100%	100%	100%	100%	100%	100%

- 23.6% (25/106) surgical cases show triple genetic alterations, for which 76% (n=19) are epithelioid and 24% (n=6) are biphasic mesotheliomas.

- Survival analysis: 93 patients with survival data with median survival 18.6 months (95% CI, 12.1-25.1).

  - Overall survival is correlated with histological subtypes, grading and *CDKN2A* homozygous deletion status.
  - BAP1 loss and *NF2* hemizygous loss are not correlate with overall survival.

Take-home message: This multi-institutional study confirmed the prior findings that *NF2* hemizygous loss by FISH is a good test in separating malignant from benign mesothelial proliferation with ~50% sensitivity and 100% specificity in both surgical and cytology specimens. Combined with the other well-established tests (BAP1 IHC + *CDKN2A* FISH), the combined sensitivity is close to 100% (98.1% in surgical and 94.4% in cytology specimens). *NF2* hemizygous loss is more often seen in epithelioid mesotheliomas and has no prognostic significance.

Kishikawa S, et al. Comprehensive Clinicopathological Characteristics and Mucin Core Proteins Expression Profiles of Bronchiolar Adenoma. Histopathology. 2023;82(2):264-275.

Purpose: There are two major forms of mucins, including membrane-bound mucins (such as MUC1 and MUC4) and secretory mucins (such as MUC5AC and MUC5B, major components of airway mucus). Pulmonary invasive mucinous adenocarcinoma frequently express MUC5AC and MUC6 but rarely MUC2 (gastric mucin phenotype). This study is to understand the mucin core protein expression profiles of bronchiolar adenoma (BA) by immunohistochemistry and to validate the clinicopathologic and molecular features in BAs.

Methods: 20 BAs (10 proximal type and 10 distal type) are identified from 2013 to 2021, including 2 patients with idiopathic interstitial pneumonia.. Immunohistochemical stains (p40, MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC6, TTF1, ALK, HNF4alpha) and NGS (including hotspot regions of 50 oncogenes and tumor suppressor genes) are performed on all 20 BAs.

Results:

- The overall clinicopathological features, including age, sex, smoking status, tumor size and tumor location, are similar in patients with proximal or distal BAs (Table 1). Interestingly, all BAs except one are in the lower lobe.

- None of the 20 BA cases had pre-operative biopsies. Among 18 cases (9 proximal and 9 distal type) underwent frozen section diagnosis, only 7 (39%) were accurately diagnosed as BA at the time of frozen section diagnosis. 5 proximal type and 4 distal type were misdiagnosed as adenocarcinoma in situ or invasive mucinous adenocarcinoma.

- NGS confirmed that *BRAF* V600E (n=7; 35%) is the most frequent mutation, followed by *KRAS* (n=6; 30%) and *EGFR* (n=3, 15%). No genetic alterations are identified in 4 proximal-type BAs (Figure 2). These mutations are mutually exclusive.

-Mucin core expression profiles

	MUC1	MUC2	MUC4	MUC5AC	MUC5B	MUC6
NML bronchiole	Focal, epithelial and basal cells	No expression	Diffuse, epithelial and basal cells	Scattered cells	Scattered cells	No expression
NML Type II pneumocyte	Diffuse	No expression	No expression	No expression	No expression	No expression
BAs (n=20)	+ (n=20, focal)	- (n=20)	+ (n=20; 18 diffuse)	+ (n=9; 45%, focal)	+ (n=20; focal)	+ (n=5; 25%, focal)
Glandular papilloma / mixed squamous cell and glandular papilloma (n=2)	+	-	+	+	+	-

- The proximal type and distal type BAs share similar mucin protein expression profiles that include common expression of MUC1, MUC4 and MUC5B, similar to the normal bronchiolar epithelium.

Take-home message: BAs show diffuse MUC4 and patchy MUC1 and MUC5B expression, similar expression pattern as normal bronchiolar epithelium. This study supports the notion that BAs are derived from the bronchiolar epithelium.

Articles for notation

Neoplastic

Logan CD, et al. National Trends in the Quality of Segmentectomy for Lung Cancer. J Thorac Cardiovasc Surg. 2023;165 (1):351-363.

Purpose: While anatomic lobectomy with mediastinal lymph node sampling has been the standard of care for patients with early-stage non-small cell lung cancer (NSCLC), segmentectomy has emerged as an acceptable approach. This study use the national cancer database (NCDB) to assess the national trends in the prevalence of segmentectomy for early-stage NSCLC, determine adherence to oncologic quality measures (including 10 or more regional lymph nodes sampled, negative (R0) resection margins, and tumor size 2 cm or less) for patients receiving segmentectomy, evaluate the difference between academic and non-academic programs in both segmentectomy prevalence and quality, and evaluate the association between segmentectomy quality and survival.

Take-home message: This study demonstrated that there is an up trending of the proportion of segmentectomy of all lung resections, increased from 3.3% in 2004 to 6.1% in 2018. Academic programs performed much better by being more likely to adhere to all the quality measures than non-academic programs. Better quality of segmentectomy is associated with better survival.

Park BJ, et al. Proposal of a revised international association for the study of lung cancer grading system in pulmonary non-mucinous adenocarcinoma: The importance of the lepidic proportion. Lung Cancer. 2023;175:1-8.

Purpose: This study is to validate the recently proposed grading system for non-mucinous lung adenocarcinoma by the international association for the study of lung cancer (IASLC) in the Asian /Korean population, given that the IASLC grading system is derived mainly from Western populations. IASLC grade 2 accounts for half of the total patients. A modified model is proposed by adding the proportion (10%) of lepidic growth as an additional consideration to further classify IASLC grade 2 adenocarcinomas into 2 subgroups, 2a (>= 10% lepidic growth) and 2b (no or < 10% of lepidic growth). The new/modified grading system is comparing to the IASLC grading system to evaluate which one shows better performance in terms of correlation with disease-free survival and overall survival.

Take-home message: The IASLC grading system has shown good performance in predicting the prognosis in Asian/Korean population. Further subclassifying IASLC grade 2 patients by considering the proportion of lepidic growth can further stratify patients into different prognostic groups. Whether similar advantage of subclassifying IASLC grade 2 patients can also be seen in the Western population needs further studies.

Zhang Y, et al. Validation of the novel international association for the study of lung cancer grading system and prognostic value of filigree micropapillary and disclosive growth pattern in invasive pulmonary adenocarcinoma. Lung Cancer. 2023;175:79-87.

Purpose: Similar to the Korean study described above, this study is to validate the recently proposed grading system for non-mucinous lung adenocarcinoma by the international association for the study of lung cancer (IASLC) in the Asian /Chinese population. This study also evaluates the effects of discohesive and filigree micropapillary patterns on disease-free survival (DFS) and overall survival (OS). Lastly, competitive risk analysis was used to understand whether these 2 patterns correlate with increased risk of recurrence and/or metastasis in stage I invasive lung adenocarcinoma.

Take-home message: This retrospective study in Asian /Chinese population confirmed the IASLC grading system can effectively stratify patients with stage I-III invasive lung adenocarcinoma in terms of DFS and OS. In patients with IASLC grade 1 and 2 adenocarcinomas, the simultaneous presence of filigree

micropapillary and discohesive patterns significantly affected DFS. For stage I patients, these 2 patterns were also risk factors for increased recurrence and metastasis.

Non-eoplastic

Humbert M, et al. 2022 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Respir J. 2023;61(1):2200879.

Take-home message: In the 2022 guideline, the mPAP threshold has been lowered to 20mm Hg (from 25 mmHg) for diagnosing pulmonary hypertension (PH) and the cut-off point for abnormal pulmonary vascular resistance (PVR) has been lowered to > 2 Wood units (previous: > 3 Wood units). The new guideline emphasizes that PAH patients with comorbid cardiopulmonary disease have worse survival and are less likely to respond well to PAH therapies. DLco has been identified as a prognostic and potential risk stratification marker in PAH. This is a comprehensive guideline with updates on classification of PH, risk stratification scheme, and clinical management.

Donahoe LL et al. Outcomes of lung transplantation from donors with a history of substance abuse. J Thorac Cardiovasc Surg. 2023;165(1):384-395.

Purpose: Traditionally, strict rules are used to only accept "ideal" donor lungs. Many donors do not qualify these strict rules. However, there are studies that show recipient outcomes are equivalent when using nonideal donors. This study is about a single institutional experience from 2013 to 2019 to determine whether donor history of substance abuse impacts lung acceptance rate and recipient outcomes (overall survival <OS> and time to chronic lung allograft dysfunction <CLAD>).

Take-home message: Donors with history of opioid overdose death or opioid use did not affect rates of lung acceptance in this single institutional study. Recipients of lungs from donor with history of substance abuse (including opioid overdose, opioid use, or marijuana use) showed similar OS and time to CLAD. Therefore, lungs from donors with a history of substance abuse should be considered for transplantation.

<u>Reviews and Editorials</u>

Martin SD, et al. Immunohistochemical demonstration of Merlin/NF2 loss in mesothelioma. *Mod Pathol*. 2023;36(1):100036.

Purpose: This study is to evaluate /validate the findings from Chapel et al. (Chapel DB et al. Mod. Pathol. 2022;35(10):1383-1397) regarding whether Merlin is a good immunohistochemical (IHC) marker to diagnose malignant mesothelial process.

Take-home message: Merlin IHC is potentially a good marker in separating malignant from benign mesothelial proliferations. Two different antibody clones of Merlin were tested. Antibody clone D1D8 (used in the study from Chapel et al) recognizes an epitope near the N terminus of Merlin and antibody clone D3S3W recognizes an epitope near the C terminus (D3S3W showed more intense staining than D1D8). Using mesothelioma tissue microarray, 35% (14/40) epithelioid mesotheliomas and 39% (7/18) sarcomatoid mesotheliomas showed loss of expression of Merlin, whereas reactive mesothelial proliferations, including both epithelial (34) and spindle cell (14) mesothelial proliferations, showed retained cytoplasmic/membrane staining for Merlin. Subclonal loss of Merlin was observed, which may reflect the fact that *NF2* loss is a late event in mesotheliomas. Not all cases with molecularly proving gene alteration of *NF2* showed loss of expression of Merlin by IHC though (16 out of 57 cases with *NF2* genetic alterations showed retained Merlin staining).

Sohn A and Moran CA. Primary mediastinal germ cell tumors. Semin Diagn Pathol. 2023;40(1):37-46.

Take-home message: This review summarizes the histopathologic, immunohistochemical and molecular features of mediastinal germ cell tumors with proposed staging system. Mediastinal germ cell tumors predominantly occur in young men with a male: female of 9:1. Teratomas represent the most common mediastinal germ cell tumor followed by pure seminomas. Diagnostic criteria are similar to its counterpart in the gonads. The diagnosis of mediastinal germ cell tumor is often limited to small mediastonoscopic biopsy, since patients are often treated with neoadjuvant therapy before resection. The resection specimen may show only therapy-related tumor necrosis, which makes further histopathological assessment impossible.