A Pulmonary Nodule with an Unexpected Mutation Profile

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CASE DESCRIPTION

A 70-year-old man presented with 3 weeks of confusion and ataxia. He had a 20 pack/year history of smoking (he quit over 10 years ago) and melanoma in situ excised 2 years ago. Upon admission, a computed tomography scan demonstrated 3 hemorrhagic brain masses and 2 right upper lobe lung nodules.

The patient underwent core biopsy and fine-needle aspiration (FNA) of a right upper lobe nodule. The core biopsy showed normal tissue, and the cytology specimen showed cuboidal cells with marked nuclear variability (Fig. 1A and B). The cell block contained fragments of atypical cells with intranuclear inclusions and hobnailing (Fig. 1C). The cytology specimen was signed out as non-small cell carcinoma, consistent with adenocarcinoma.

Molecular profiling was performed using an in-house targeted DNA sequencing panel (GatewaySeq), which is routine for new diagnoses of lung adenocarcinoma at our institution. GatewaySeq is a tumor-only hybridization capture-based next-generation sequencing (NGS) assay that targets single nucleotide variants and small insertions and deletions in 133 genes, copy number alterations in 11 genes, and rearrangements involving 8 genes in addition to microsatellite status and tumor mutational burden (TMB). Testing identified 6 oncogenic/likely oncogenic variants: *BRAF* p.Glu501Lys, *BRCA1* p.Val1838Gly, *KIT* p.Val559Asp, *TERT* c.-126_-124delinsTTT, *TERT* c.-126_-124delinsTCT, and *TP53* p.Gln331Ter (Fig. 2A). In addition, 54 variants of uncertain significance were detected in 31 genes. TMB was 94 mutations per megabase (mut/Mb). Fifty of the 60 total variants identified were C > T transitions, and mutational signature analysis revealed a type SBS7a signature, a mutation profile associated with ultraviolet (UV) radiation (Fig. 2B).

QUESTIONS TO CONSIDER	
1.	What are the most common malignant etiologies of a pulmonary nodule?
2.	What are the most common driver mutations in patients with lung adenocarcinoma?
3.	How should clinical sequencing results that seem incompatible with the stated diagnosis be handled?
4.	How can mutational signature analysis inform the interpretation of clinical sequencing results?

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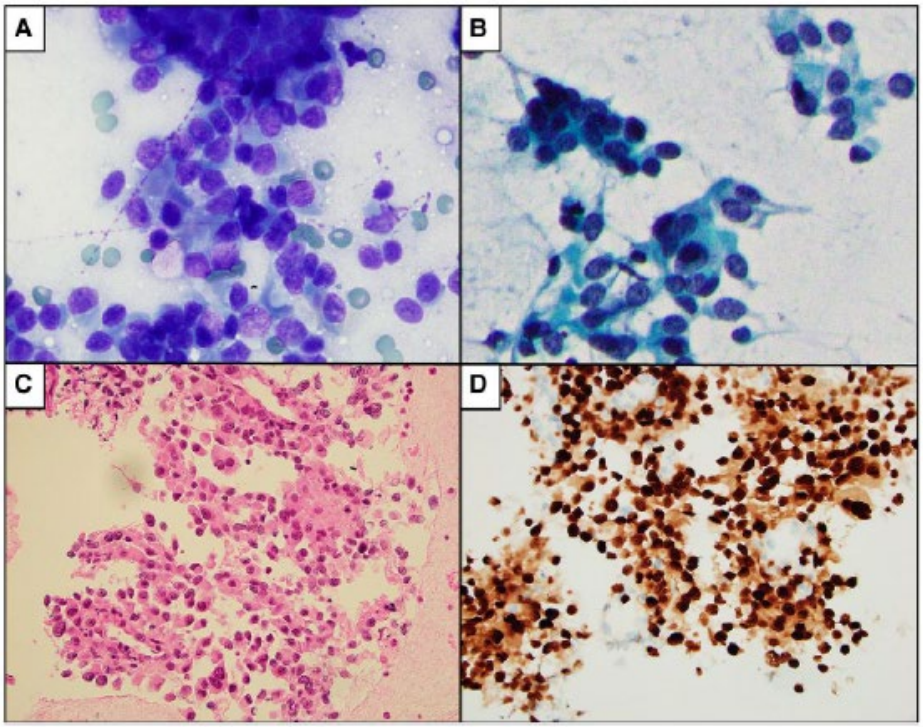

Fig. 1. Morphology and selected stains from neoplastic cells. (A), Diff-Quik-stained direct smears highlight a relatively uniform population of dyscohesive cells with round to oval nuclei, small nucleoli, and small nuclear inclusions. The cytoplasm is amphophilic without pigment. (B), Papanicolaou-stained direct smears show a relatively uniform population of cells. Nuclei are not significantly larger than bronchial epithelial cells and nucleoli are prominent. (C), Hematoxylin and eosin-stained cell block shows fragments of alveolar type tissue with a population of dyscohesive cells; the hobnail appearance is reminiscent of a well-differentiated adenocarcinoma. (D), SOX10-immunostained cell block has diffusely positive nuclear staining in the malignant cells. Color figure available at https://academic.oup.com/clinchem.

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Gene	Transcript	Coding Change	Protein Change	Variant Allele Fraction (%)
BRAF	ENST00000646891.2	c.1501G>A	p.Glu501Lys	21
BRCA1	ENST00000357654.9	c.5513T>G	p.Val1838Gly	23
KIT	ENST00000288135.6	c.1676T>A	p.Val559Asp	54
TERT	ENST00000310581.10	c.-126_-124delinsTTT	—	11
TERT	ENST00000310581.10	c.-126_-124delinsTCT	—	22
TP53	ENST00000269305.9	c.991C>T	p.Gln331Ter	27

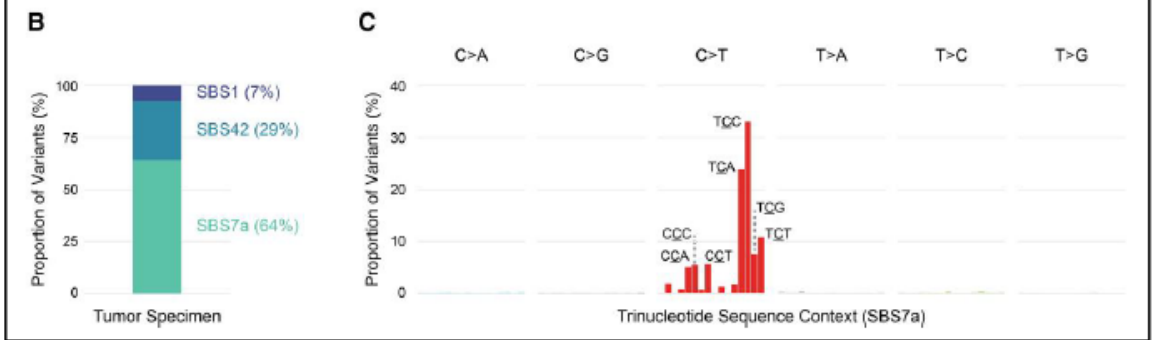

Fig. 2. Variant details and mutational signature analysis. (A), Detailed annotations for likely oncogenic/oncogenic variants identified in this case. (B), Decomposition of the observed variant profile based on re-fitting known mutational signatures supported the presence of SBS7a, SBS42, and SBS1. (C), Reference variant frequencies for the SBS7a mutational signature. The frequency of each single-nucleotide substitution (C > A, C > G, ..., T > G) for each trinucleotide context (ANA, ANC, ..., TNT) in this signature is plotted on the y-axis. The variants that comprise SBS7a are predominantly C to T transitions occurring after C or T nucleotides, especially TCA > TTA or TCC > TTC. Color figure available at https://academic.oup.com/clinchem.

Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the March 2025 issue of *Clinical Chemistry*. To view the case and comments online, go to https://academic.oup.com/clinchem/issue/71/3 and follow the link to the Clinical Case Study and Commentaries.

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