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Case Report

Sequential triple primary malignancies in the larynx, lung, and liver with discordant p53 expression patterns: a case report

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Abstract: Sequential triple primary malignancies are very uncommon. Distinguishing new primary tumors from metastases in cancer survivors is a diagnostic challenge. This case illustrates how comprehensive immunohistochemistry (IHC), particularly p53 expression patterns as a surrogate for TP53 gene status, can determine clonal independence. A 71-year-old male ex-smoker presented with three malignancies over 4 years. First, laryngeal squamous cell carcinoma (pT1bN0M0) showed complete loss of p53 staining (null pattern, truncating mutation). Four years later, lung adenocarcinoma (pT1bN1M0; TTF-1+/Napsin A+) showed strong, diffuse p53 nuclear positivity (missense mutation pattern). One month later, intrahepatic cholangiocarcinoma (pT1N0M0; CK7+/CK19+/TTF-1-) showed wild-type p53 (scattered weak positivity). Divergent p53 IHC patterns (two mutant phenotypes and one wild-type) indicated three independent primaries. The patient recovered well after each curative-intent surgery. Meticulous IHC profiling and careful interpretation of p53 staining patterns are crucial for distinguishing multiple primary cancers from metastatic disease.

Keywords: Case report, multiple primary malignancies, p53 protein, immunohistochemistry, laryngeal neoplasms, lung neoplasms

Introduction

Multiple primary malignancies (MPMs) are defined as two or more distinct cancers in one patient. Their incidence has increased due to improved diagnostics and longer survival. Triple primary cancers are rare (<1% of MPMs) [1]. A key clinical challenge is differentiating a new primary from metastasis, which changes management from palliative to curative.

Histology and immunohistochemistry (IHC) are fundamental to diagnosis. p53 IHC pattern serves as a surrogate for TP53 gene status [2, 3]. Complete loss (null pattern) suggests truncating mutations; strong diffuse nuclear positivity indicates missense mutations; wild-type pattern shows scattered variable staining. Discordant p53 patterns among tumors support independent clonal origins.

Most literatures about MPMs are on double primary cancers. Data on triple primaries, especially those based on detailed analysis of the p53 pattern to infer clonal relationships, are scarce. Herein, we report a rare case of sequential triple primary malignancies (larynx, lung, liver) in a heavy smoker, highlighting the diagnostic value of p53 IHC patterns.

Case presentation

A 71-year-old Chinese male ex-smoker (40 pack-years) presented with hoarseness in December 2020. Laryngoscopy showed bilateral vocal cord lesions. Microlaryngeal excision revealed severe dysplasia and carcinoma in situ. CO_2 laser partial laryngectomy confirmed moderately-poorly differentiated squamous cell carcinoma, pT1bN0M0 (**Figure 1A**). IHC showed complete p53 loss (null pattern, truncating mutation) (**Figure 1B**).

Routine follow-up in January 2025 revealed new bilateral pulmonary nodules (**Figure 2A**, **2B**). Contrast-enhanced CT showed a 1.8 cm

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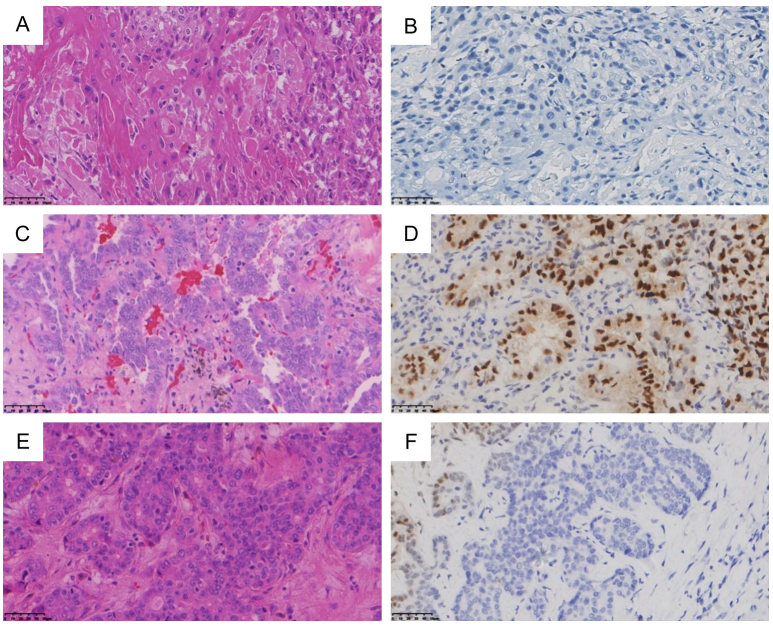

Figure 1. Histopathological and immunohistochemical features of the three sequential primary malignancies. A. Laryngeal SCC (H&E). B. p53 null pattern. C. Lung adenocarcinoma (H&E). D. p53 diffuse strong positivity (missense pattern). E. Intrahepatic cholangiocarcinoma (H&E). F. p53 wild-type pattern (scattered weak positivity). (scale bar = 50 μm, original magnification: 400×).

solid nodule in the right lower lobe (**Figure 2C**, **2D**). Video-assisted thoracoscopic lobectomy with lymph node dissection was performed. Pathological examination showed a single focus of poorly differentiated invasive adenocarcinoma (acinar predominant, 2.1 cm) (**Figure 1C**), positive for CK7, TTF-1, BRAF V600E, EGFR, Napsin A, and negative for P40, CK5/6, ALK. p53 showed strong diffuse nuclear positivity (missense mutation pattern) (**Figure 1D**). One of three lymph nodes (station 10) had micrometastasis (<2 mm), final stage pT1b-N1M0.

One month later, abdominal ultrasound revealed a hypoechoic lesion in liver segment IV. Contrast-enhanced MRI showed arterial hyperenhancement with delayed washout, suspicious for primary hepatic malignancy (**Figure 2E**, **2F**). Laparoscopic segmentectomy with cholecystectomy was performed. The histopathological findings of the hepatic resection specimen showed moderately differentiated adenocarcinoma with small ductular pattern, CK7+/CK19+, negative for CDX2, CD10, TTF-1, Napsin A, HepPar-1, and Arg-1, confirming intrahepatic cholangiocarcinoma (small duct type) (**Figure 1E**). p53 showed wild-type pattern (scattered weak positivity) (**Figure 1F**). The final pathological stage was pT1N0M0.

He is being followed up on our oncology hospital of diversified treatment daily basis. At the last follow-up 6 months post-hepatectomy, there was no recurrence of disease and the patient maintained a good performance status and quality of life.

Discussion

Distinguishing whether a new primary malignancy is a new primary malignancy in cancer survivors is a difficult diagnosis with important implications for treatment [4]. As double primary cancers are becoming more common, sequential triple primary cancers are very rare. This case is not only rare but also comprehensively demonstrates the entire diagnostic process, especially the fine interpretation of p53 IHC patterns.

The diagnosis followed the established clinical criteria, based on the different histology (squamous, glandular, and ductular), and confirmed the line through IHC. The pulmonary tumor was positive for TTF-1 and Napsin A, the hepatic tumor expressed CK7, CK19, and was negative for TTF-1, thus excluding metastasis from the lung primary. This exclusion was corroborated by the composite profile of the hepatic tumor: it had a glandular/ductular appearance and was positive for biliary markers (CK7/CK19), but negative for pulmonary markers (TTF-1/Napsin A), and showed a wild-type p53 pattern. And so this conclusive pathologic diagnosis moved the management from palliation of systemic therapy to resect cure intent.

It should be noted that p53 IHC is a proxy for TP53 mutation status. Discordant patterns provide strong supporting evidence for clonal independence, but the ultimate proof would need to be obtained through comprehensive genomic sequencing. In this case, p53 IHC revealed a trinity of different patterns: p53 mu-

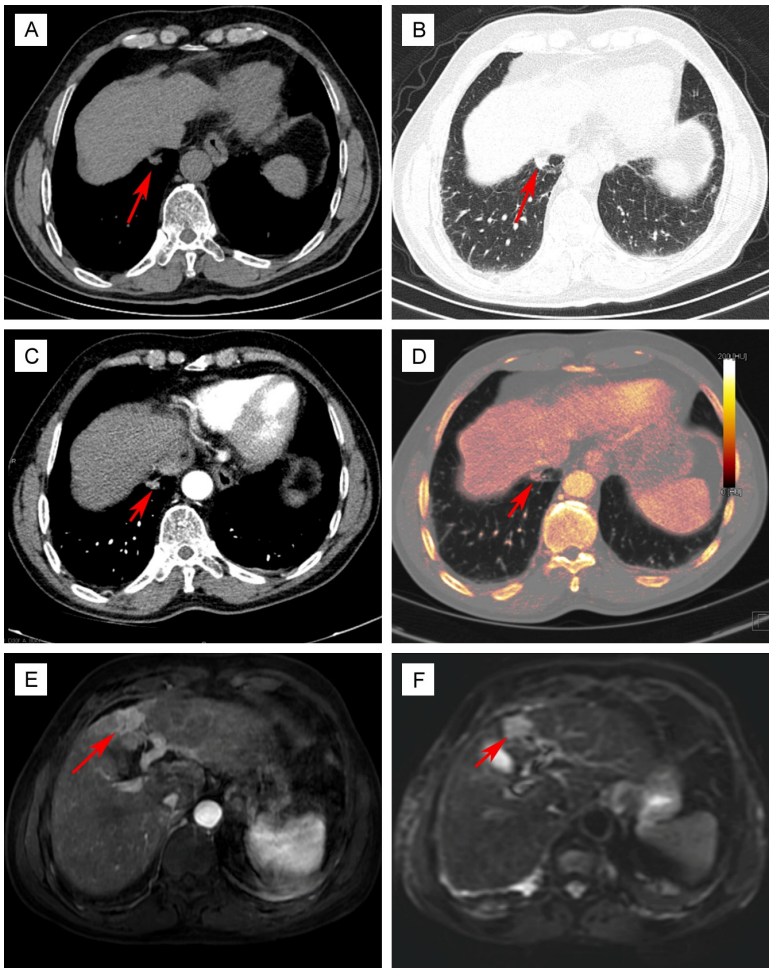

Figure 2. Thoracic and abdominal imaging findings. A, B. Surveillance chest CT scan (January 2025) depicting multiple small bilateral pulmonary nodules, with a new irregular solid nodule in the right lower lobe (arrows). C, D. Contrast-enhanced chest CT (arterial phase) of the same patient confirms the suspicious, enhancing right lower lobe nodule (arrows), radiologically concerning for primary lung malignancy. E, F. Contrast-enhanced abdominal MRI (arterial phase) demonstrates a hyperenhancing lesion in segment IV of the liver (arrow), suggestive of a primary hepatic malignancy.

tant (truncating) in the laryngeal carcinoma; a strong, diffuse pattern of the mutant (missense) in the lung adenocarcinoma; a wild-type pattern in the intrahepatic cholangiocarcinoma. This finding of two distinct mutant p53 phenotypes in addition to a wild-type pattern offered strong additional support of three independent clonal origins and suggested three different molecular pathogenesis [5].

There are three different statuses for p53 among these three tumors, and three distinct pathogenic mechanisms. Null pattern in larynx and mutant pattern in lung both point out direct, but mechanistic disruptions of p53 [6, 7]. The lymph node metastasis in the lung tumor may be associated with the specific mutant p53 phenotype [8]. As opposed to the wild-type p53 pattern in the liver tumor, which indicates development by p53-independent routes. Positive IHC screening for BRAF V600E (VE1 clone) and EGFR (total protein) in the lung tumor was not verified by subsequent PCR-based molecular tests, indicating that IHC is a surrogate for mutation status and genetic testing should be done to confirm. This molecular heterogeneity implies that even the same person has a complex carcinogenesis.

A clear temporal sequence was established (**Table 1**): laryngeal carcinoma (2020) first, then lung adenocarcinoma (2025), and finally intrahepatic cholangiocarcinoma (2025). This sequencing argument is opposed to synchronous metastasis. A large amount of causality can be supported by the patients being quite a bit of a long-term smoker [9], which is well known as a multi-organ carcinogen in all three cancers. Supports field cancerization theory that widespread epithelial exposure results in independent malignancies, more reasonable explanation for atypical metastasis.

The pathologic picture of each change was intercommunication and practice. Confirmation of three independent primary cancers, each at an early stage (pT1), was the unambiguous basis for performing a sequence of curative-intent surgical resections, namely a laryngectomy, followed by a right upper lobe lobectomy, and then a hepatectomy. This approach is based on the most precise histology and IHC, whereas the palliative chemotherapy or immunotherapy which is generally used when you

Table 1. Clinicopathological characteristics and immunohistochemical profiles of the three primary malignancies

Characteristics	Tumor 1: Larynx (2020.12)	Tumor 2: Lung (2025.2)	Tumor 3: Liver (2025.3)
Histologic Type	Squamous cell carcinoma	Adenocarcinoma	Adenocarcinoma (cholangiocarcinoma)
pTNM Stage	pT1bN0M0	pT1bN1M0	pT1N0M0
Surgical Procedure	Partial laryngectomy	VATS lobectomy	Liver segmentectomy
IHC (+) Markers	Not performed*	TTF-1; Napsin A; CK7; BRAF V600E; EGFR	CK7; CK19
IHC (-) Markers	Not performed*	P40; CK5/6; ALK	TTF-1; Napsin A; CDX2; CD10; HepPar-1; Arg-1
p53 IHC Pattern	Null pattern (truncating mutation)	Missense mutation pattern (Strong, diffuse positivity)	Wild-type pattern (scattered, weak positivity)

*Note: tumor has classic squamous morphology, and additional IHC was deemed unnecessary. The p53 staining for this tumor, along with the comprehensive IHC profiles presented in this table, was performed retrospectively on archival tissue for the purpose of this comparative study.

assume that you have metastatic disease would be inappropriate here. This case indicates that in surgical oncology, diagnosis by pathologist is not a byproduct but the basis of plans to treat.

The results thereupon are not alike necessarily. Key variables include stage of each new primary found, the functional status and comorbidities of the patient, and access to specialized multidisciplinary care. And we got the favorable outcome because all of those things lined up.

This case report has its own deficiencies. First of all, as a single retrospective report, the conclusions cannot be widely applied. Second, the lack of a complete genetic profile (such as next-generation sequencing) results in no genetic level evidence of clonality, only using strong surrogate evidence of IHC. And the third, the follow up durations (6 mos. from the last cut out) to reach oncological outcomes. These defects are also its main diagnostic and educational value and point out the way to follow the research in the future.

And in the future for complicated multiple primaries, we would perhaps do more molecular profiling, that is something I'll go forward. Liquid biopsy approach like circulating tumor DNA analyses is hopeful direction to detecting minimal residual disease as well as second primaries at earlier stage [10].

This case presents a very rare and detailed case of three primary cancers in sequence. Its unique merit can be attested to on the organ sequence, the improved diagnostic capability via the combo of a pathology and an IHC, the instructional application of discordant p53 expression pattern. We recommend: (1) Pathological verification of all new lesions in cancer survivors should be required before attributing them to metastatic disease; (2) IHC (specific lineage marker + p53, etc.) may be considered only after giving careful thought to the pattern of staining (null, diffuse, wild-type); And (3) Intensify surveillance in high-risk patients, such as heavy smokers with a history of aerodigestive cancer, so that subsequent primaries can be detected early. This case suggests us that no matter how complex case with the appearance of sequence primary multiple malignancy, if we can correctly diagnose it and adaptively treat according to different stages of disease occurrence, we could also obtain good results.

Conclusion

This is a very rare case to prove that comprehensive immunohistochemical detection is needed to distinguish multiple primary cancers from metastases. Identify 3 p53 expression pattern (null, diffuse mutant, wild -type) was strongest piece of evidence of separate independent clonal beginning. So we can see when you do have an exact diagnosis you can have a good outcome just from local high pressure for complex presentation with a sequence of multiple primaries.

Disclosure of conflict of interest

None.

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