Pathology Casebook: Microscopy to Molecular Correlations in Disease Diagnosis

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Introduction

Pathology sits at the nexus of clinical medicine and the basic sciences, translating cellular and molecular alterations into diagnoses that guide patient care. This book was conceived to bridge the span from the glass slide to the gene panel, showing how histology, immunohistochemistry, and molecular testing inform and refine each other. By anchoring every chapter in a real-world case, we place the reader at the microscope and in the tumor board simultaneously, emphasizing practical decision points, common pitfalls, and the downstream impact of each conclusion.

Each case unfolds through step-by-step diagnostic reasoning. We begin with the clinical vignette and key radiologic or gross features, proceed to the microscopic patterns that define the differential diagnosis, and then build an evidence-based ancillary testing strategy. Rather than offering exhaustive lists, we prioritize focused panels—special stains, immunostains, in situ hybridization, FISH, PCR, and next-generation sequencing—selected for their ability to confirm lineage, subclassify disease, or reveal actionable biomarkers. Along the way, we highlight pre-analytic variables, specimen adequacy, and tissue stewardship to ensure that small samples yield maximal information.

Molecular results are most powerful when interpreted in context. Throughout the chapters, mutations, fusions, copy-number changes, methylation profiles, and signatures are integrated with morphology and immunophenotype to produce unified, standards-aligned diagnoses. We discuss variant interpretation frameworks, clonality assessment, and orthogonal confirmation, while drawing attention to patterns that are specific, sensitive, or merely suggestive. When results conflict, we model how to reconcile discordant data by revisiting the slide, expanding the differential, and considering technical artifacts.

Because diagnosis is not an end in itself, every case closes the loop to patient management. We outline prognostic stratification and therapeutic implications, from risk categories in hematologic neoplasms to predictive biomarkers in solid tumors. The emphasis is on how pathology informs clinical choices—surgery, targeted therapy, immunotherapy, and surveillance—while acknowledging uncertainty, evolving evidence, and the necessity of multidisciplinary collaboration. Practical reporting language, reflex testing pathways, and cost-conscious algorithms are offered where they can genuinely improve care.

This book is written for practicing pathologists, trainees across disciplines, and clinicians who want a deeper understanding of disease mechanisms. Novices will find clear frameworks for approaching common and challenging entities, while experienced readers can use the cases as compact refreshers and teaching material. Visual learners will appreciate annotated photomicrographs and schematics that link what is seen under the microscope to what is detected on the sequencer or probe.

Finally, we recognize that the field is dynamic. New assays appear, classification schemes are refined, and therapeutic landscapes shift. The enduring value of this casebook lies not only in its curated examples, but in the transferable habits of mind it cultivates: precise observation, disciplined hypothesis generation, judicious use of ancillary studies, and transparent, clinically meaningful synthesis. Our aim is that, case by case, the reader will strengthen the bridge from microscopy to molecules—and, in doing so, deliver clearer answers to the clinical problems that matter most.

CHAPTER ONE: The Solitary Pulmonary Nodule: From Lepidic Pattern to EGFR Exon 19 Deletion

The call came in on a Tuesday morning, a familiar scenario playing out in pathology departments worldwide. Dr. Evans, a seasoned pulmonologist, was on the line with a query about a recent CT scan. His patient, a 62-year-old former smoker with a lingering cough, had presented with a solitary pulmonary nodule—a 1.8 cm, well-defined lesion in the right upper lobe, incidentally discovered during a workup for his chronic obstructive pulmonary disease. The radiologist had flagged it as indeterminate, recommending further investigation. This is often where our journey begins, a tiny shadow on an imaging plate holding the potential for a myriad of diagnoses, from benign granuloma to aggressive malignancy.

The clinical presentation of a solitary pulmonary nodule (SPN) is inherently nonspecific, often discovered incidentally as in this case. Patients may be

asymptomatic, or present with symptoms attributable to their underlying conditions, such as the chronic cough in our patient. The diagnostic challenge lies in efficiently and accurately differentiating between benign and malignant etiologies to guide appropriate patient management. Factors such as patient age, smoking history, nodule size, growth rate, morphology (e.g., spiculation, cavitation), and PET avidity all contribute to the pre-test probability of malignancy. For Dr. Evans' patient, the size and the patient's smoking history nudged the probability meter firmly towards the 'suspicious' end of the spectrum.

Given the clinical context and the radiologic findings, Dr. Evans opted for a CT-guided fine-needle aspiration (FNA) biopsy. This minimally invasive procedure is frequently employed for accessible lesions, offering a balance between diagnostic yield and patient risk. The pathologist's role begins even before the slides arrive in the lab, with careful attention to pre-analytic factors. Specimen adequacy is paramount in FNA biopsies, particularly when molecular testing is anticipated. Rapid on-site evaluation (ROSE) by a cytopathologist can be invaluable, confirming the presence of diagnostic material and triaging samples for ancillary studies, thereby minimizing the need for repeat procedures. In this instance, ROSE confirmed cellular material was indeed present, and a portion of the aspirate was collected for cell block preparation and potential molecular studies.

The initial microscopic examination of the Papanicolaou-stained smears and H&E-stained cell block sections revealed a proliferation of atypical epithelial cells. These cells displayed moderate anisonucleosis, irregular nuclear contours, and prominent nucleoli. They were arranged in small clusters and singly, some exhibiting a somewhat discohesive pattern. The background showed evidence of chronic inflammation and scattered alveolar macrophages, consistent with the patient's smoking history and underlying lung disease, but these features did not explain the presence of the atypical cells. The immediate differential diagnosis for atypical epithelial cells in a pulmonary FNA is broad, ranging from reactive atypia to various forms of lung carcinoma.

One of the first considerations was whether these atypical cells represented a primary lung adenocarcinoma. Adenocarcinomas are the most common type of lung cancer and can present with diverse morphologic patterns. The challenge here was to distinguish a well-differentiated adenocarcinoma from other benign processes that might mimic malignancy, as well as from other types of lung cancer, such as squamous cell carcinoma or metastatic disease. The cell block preparation became particularly important at this juncture, allowing for the application of immunohistochemistry (IHC).

IHC is a cornerstone of diagnostic pathology, leveraging antibody-antigen interactions to identify specific proteins within cells and tissues, thereby aiding in tumor classification and prognostication. For a suspicious pulmonary lesion, a standard

immunohistochemical panel typically includes markers for epithelial differentiation, such as cytokeratins (e.g., CK7, CK20, TTF-1), and lineage-specific markers like TTF-1 (thyroid transcription factor-1) and napsin A for adenocarcinoma, or p40 and CK5/6 for squamous cell carcinoma. Other markers, such as synaptophysin and chromogranin, might be used to rule out neuroendocrine tumors, while a broad-spectrum cytokeratin (e.g., AE1/AE3) confirms epithelial origin.

In our case, the atypical cells showed strong and diffuse nuclear positivity for TTF-1 and cytoplasmic positivity for napsin A. This immunoprofile is highly characteristic of adenocarcinoma of pulmonary origin, effectively narrowing the differential diagnosis and pointing towards a primary lung malignancy. The cells were negative for p40 and CK5/6, ruling out squamous cell carcinoma. This strategic application of IHC significantly refines the diagnosis, providing crucial information about the tumor's lineage and origin, even from a small biopsy sample.

With the diagnosis of adenocarcinoma established, the next critical step involved subtyping the tumor based on its architectural patterns. The 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classification of lung adenocarcinoma introduced a new paradigm, emphasizing the predominant histologic pattern observed in resected specimens, with implications for prognosis. While FNA biopsies often provide limited architectural information, the cell block can sometimes offer clues. In this case, careful examination of the cell block revealed small clusters of atypical cells lining alveolar septa without stromal invasion—a pattern suggestive of lepidic growth.

The lepidic pattern, characterized by tumor cells growing along pre-existing alveolar structures, is a hallmark of certain well-differentiated adenocarcinomas. Tumors with a predominant lepidic pattern are associated with a better prognosis compared to those with invasive patterns such as acinar, papillary, micropapillary, or solid growth. The recognition of a predominant lepidic pattern, even in a small biopsy, can be diagnostically significant, providing early insights into the tumor’s likely behavior. However, it’s important to remember that biopsies only capture a small snapshot of the tumor, and the final architectural pattern is best assessed in a resected specimen.

The multidisciplinary tumor board, reviewing the case, acknowledged the diagnostic progress. The patient had adenocarcinoma of pulmonary origin, likely with a significant lepidic component. However, the conversation quickly shifted to treatment options. The landscape of lung cancer therapy has been revolutionized by the advent of targeted therapies, which specifically attack cancer cells expressing certain molecular alterations. For non-small cell lung cancer (NSCLC), particularly adenocarcinoma, testing for driver mutations is now standard practice, profoundly influencing treatment decisions. The most commonly tested genes include *EGFR* (epidermal growth factor receptor), *ALK* (anaplastic lymphoma kinase), and *ROS1* (ROS proto-oncogene 1, receptor tyrosine kinase).

The presence of activating mutations in the *EGFR* gene is a particularly important biomarker. *EGFR* mutations are found in approximately 10-15% of NSCLC patients in Western populations and up to 50% in East Asian populations. Patients with *EGFR* mutations are highly responsive to *EGFR* tyrosine kinase inhibitors (TKIs), which offer significantly improved progression-free survival compared to traditional chemotherapy. The two most common types of *EGFR* mutations are exon 19 deletions and L858R point mutations in exon 21, accounting for about 90% of all *EGFR* mutations. Given the strong clinical implications, molecular testing for *EGFR* mutations was requested on the cell block from the FNA.

Molecular testing on biopsy specimens, especially small ones, requires meticulous planning and tissue stewardship. The pathology department must ensure that sufficient diagnostic material is preserved for all necessary tests, prioritizing molecular studies when clinically indicated. Various molecular techniques can be employed, including real-time PCR, Sanger sequencing, next-generation sequencing (NGS), and fluorescence in situ hybridization (FISH). For *EGFR* mutation testing, PCR-based methods are frequently used due to their sensitivity and ability to detect specific mutations.

The molecular pathology laboratory received the cell block and extracted genomic DNA. A multiplex PCR assay, specifically designed to detect common *EGFR* exon 19 deletions and the L858R mutation in exon 21, was performed. The results arrived a few days later: positive for an *EGFR* exon 19 deletion. This finding was a game-changer for the patient. It meant that he was a prime candidate for targeted therapy with an *EGFR* TKI, potentially sparing him from the more generalized toxicities of conventional chemotherapy.

The *EGFR* exon 19 deletion identified in this case is considered an activating mutation, leading to constitutive activation of the *EGFR* signaling pathway, which promotes cell growth, proliferation, and survival. *EGFR* TKIs work by blocking the activity of this mutated receptor, effectively shutting down the aberrant signaling and inhibiting tumor growth. The choice of specific TKI often depends on the exact mutation and prior treatment history, with several generations of TKIs now available, each with distinct profiles and efficacy against different resistance mechanisms.

The journey from a blurry shadow on a CT scan to a precise molecular diagnosis highlights the evolving role of the pathologist. It's no longer solely about descriptive morphology; it's about integrating multiple layers of information—clinical, radiological, histological, immunohistochemical, and molecular—to arrive at a comprehensive diagnosis that directly informs patient management. This integrated approach, moving from the microscopic to the molecular, ensures that patients receive the most personalized and effective treatments available.

For our patient, the diagnosis of adenocarcinoma with an *EGFR* exon 19 deletion meant a pathway to targeted therapy. Dr. Evans discussed the findings with him, explaining how this specific genetic alteration made his tumor susceptible to a particular class of drugs. The patient, initially anxious about the prospect of chemotherapy, was visibly relieved to learn about a potentially less toxic and more effective treatment option. This underscores the profound impact of accurate and comprehensive pathology reporting on patient care and quality of life.

The case also serves as a reminder of the importance of continuous learning and adaptation in the field of pathology. The rapid pace of discovery in molecular oncology demands that pathologists stay abreast of new biomarkers, testing methodologies, and their clinical implications. The ability to interpret complex molecular reports, correlate them with morphologic findings, and communicate them effectively to clinicians is an essential skill in modern pathology.

The solitary pulmonary nodule, once a purely morphologic diagnostic dilemma, has transformed into a complex interplay of clinical risk assessment, sophisticated imaging, nuanced histology, and advanced molecular diagnostics. Each step in this diagnostic process, from the initial biopsy to the final molecular report, adds a layer of precision, ultimately guiding therapeutic decisions and improving patient outcomes. This case exemplifies the power of a multidisciplinary approach, where every piece of the puzzle contributes to a clearer picture, illuminating the path forward for the patient.
