Educational Case

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Educational Case: Non-Small Cell Lung Cancer: Pathologic Diagnosis and Molecular Understanding

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see http://journals.sagepub.com/doi/10.1177/2374289517715040.

Keywords
pathology competencies, organ system pathology, respiratory, lung neoplasia, lung, adenocarcinoma, epidermal growth factor receptor, targeted therapy, molecular medicine

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Primary Objective

Objective RS3.4: Genetics of Lung Cancer. Describe the contribution of specific genetic mutations that are found in particular lung cancers and explain how these mutations affect therapeutic decisions.

Competency 2: Organ System Pathology; Topic: Respiratory System (RS); Learning Goal 3: Lung Neoplasia.

Secondary Objectives

Objective CYP1.1: Obtaining the Specimen. Compare and contrast the 3 basic methods to obtain cytologic material for diagnosis, describe the settings in which these can be used to diagnose benign and malignant conditions, and discuss the limitations of each.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic: Cytopathology (CYP); Learning Goal 1: Cytologic Diagnosis.

Objective RS3.2: Morphologic Features of Lung Neoplasms. Discuss key gross and histopathologic features that may help differentiate between small cell, adenocarcinoma, and squamous cell carcinoma.

Competency 2: Organ System Pathology; Topic: Respiratory System (RS); Learning Goal 3: Lung Neoplasia.

Patient Presentation

A 63-year-old female presented to her primary care physician with dyspnea, fatigue, and weight loss of 20 lbs in the past 3 months. Vital signs were stable. Physical examination reveals wheezing in the right upper chest. A fixed, firm, and nontender supraclavicular lymph node was palpable. The patient had no significant past medical history. She was a nonsmoker and described a history of social alcohol use. There was no family history of cancer.

Diagnostic Findings, Part I

Chest X-ray revealed a 5-cm opacity in the right upper lung field. Differential diagnoses included pneumonia, tuberculosis, and possible malignancy. A chest computed tomography (CT) showed a solitary speculated 4.5-cm radiodense mass suspicious for malignancy.

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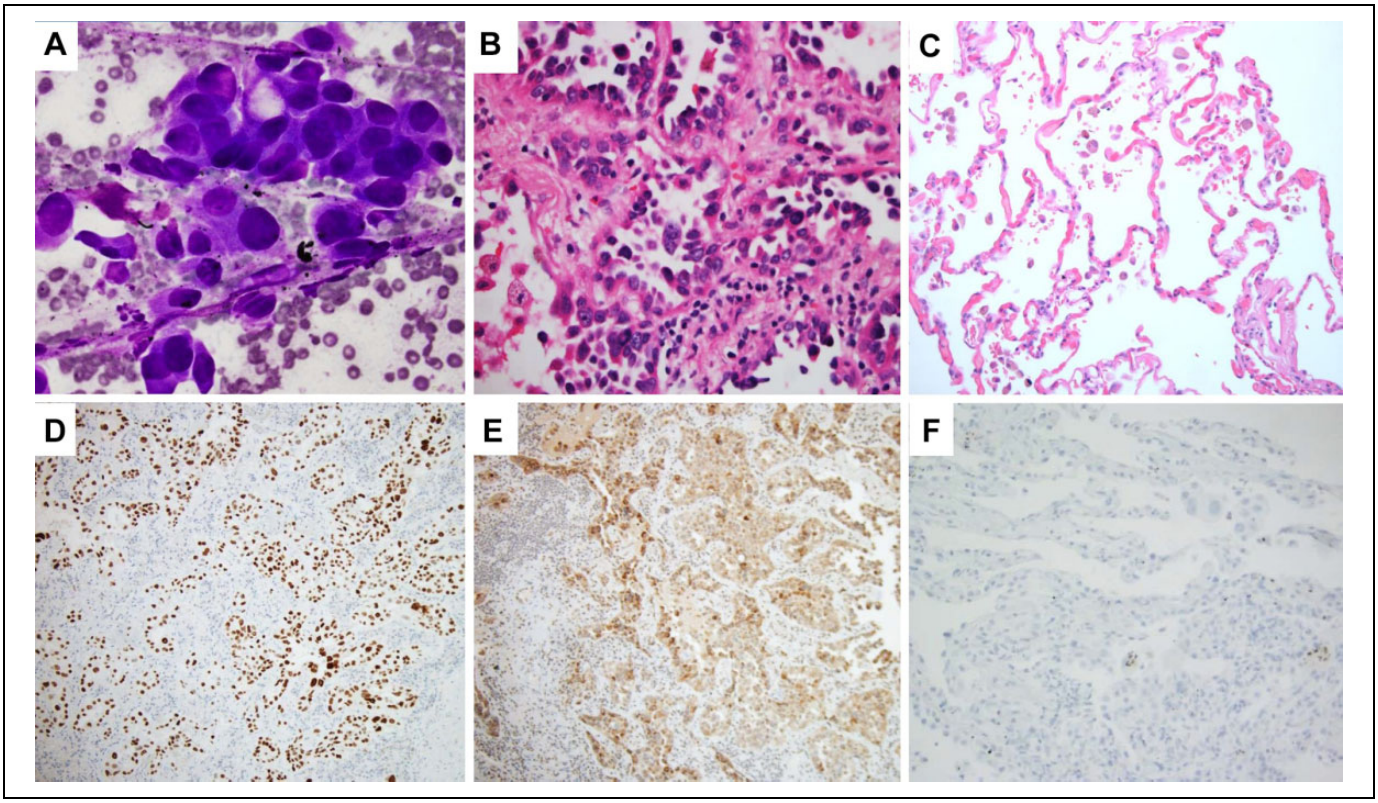

Figure 1. A, Cytology (fine needle aspiration [FNA]) findings of the patient's lung nodule (magnification ×600). The image shows a cluster of large cells with 3-dimensional structure vaguely forming an apparent glandular shape. B, Histology (core needle biopsy) findings of the patient's lung nodule. Note the malignant cells lining glandular spaces and thickened alveolar septa (magnification ×400). C, Histology of normal lung showing thin alveolar spaces line by small flattened pneumocytes. Few scattered intra-alveolar macrophages are noted (magnification ×200). D, Immunohistochemistry findings of TTF-1 showing nuclear positivity (×200). E, Immunohistochemistry of Napsin-A in this patient's tumor showing granular cytoplasmic positivity (×200). F, Immunohistochemistry of P40 in this patient's tumor is negative (×200).

Questions/Discussion Points, Part I

What Is the Best Next Step in the Evaluation of the Lung Nodules?

After a lung nodule is identified on chest imaging and a possible malignancy is suspected, it is necessary to obtain cellular material for evaluation. Often a sputum sample may be the easiest to obtain; however, while it is a noninvasive method to obtain cellular material, its sensitivity in detecting malignancy is quite low when compared to other more invasive techniques. With the help of bronchoscopy, different types of specimens, including transbronchial fine needle aspiration (FNA), aspiration washing, brushing, and bronchoalveolar lavage (BAL) could be utilized in a less invasive fashion to obtain cellular material. Bronchoscopy allows direct visualization of the tracheobronchial tree and is an ideal method to directly sample suspicious nodules near the central region.

Transbronchial FNA is a diagnostic modality that greatly augments the diagnostic accuracy of bronchial washings, brushings, and endoscopic biopsies. In the FNA procedure, a suspicious lesion is aspirated with a retractable needle ("Wang needle") which is passed through a flexible catheter sent down the bronchoscope.[1] Fine needle aspiration could also be performed with the help of ultrasound (endobronchial ultrasound-guided FNA [EBUS-FNA]). Peripheral lesions can be better sampled with percutaneous CT-guided FNA.

Diagnostic Findings, Part II

The patient was evaluated by a pulmonologist who performed an EBUS-FNA. The specimen was immediately evaluated by a cytopathologist present in the ultrasound suite. The prepared slides demonstrated malignant cells present in small 3-dimensional clusters with increased nuclear to cytoplasmic ratio and vacuolated cytoplasm (Figure 1A) consistent with non-small cell lung cancer (NSCLC). The cytopathologist also recommended a core biopsy to be taken for additional studies.

Questions/Discussion Points, Part II

What Are the Major Subtypes of Lung Cancer?

The term lung cancer, or bronchogenic carcinoma, refers to malignancies that originate in the airways or pulmonary parenchyma. Approximately 95% of all lung cancers are classified

as either small-cell lung cancer (SCLC) or NSCLC. For NSCLC, the first line of treatment is generally surgery for early-stage or localized tumors. For SCLC, on the other hand, the first-line therapeutic options revolve primarily around chemotherapy, since the tumor cells are generally considered to have metastasized at the time of diagnosis. This distinction between SCLC and NSCLC is required for proper staging, treatment, and prognosis. There are several rarer tumor types that arise in the lung and comprise only about 5% of malignancies arising there.

Non-small cell lung cancer may be further classified into a few histologic subtypes: adenocarcinoma, squamous cell carcinoma, large-cell (undifferentiated) carcinoma, and other less common subtypes including adenosquamous carcinoma and sarcomatoid carcinoma.[2]

Since the first line of treatment for all the subtypes of early-stage or localized NSCLC was the same historically, the subclassification of NSCLC was not always required for treatment purpose. Recently, however, advances in our understanding of the molecular oncogenesis and therapeutic responses have required further subclassification.

How Do We Determine the Subtype of Non-Small Cell Lung Cancer?

Much of the time, cytological features provide the first clues to the diagnosis of carcinoma. Adenocarcinoma is a type of NSCLC that may arise in the bronchi, bronchioles, or alveolar cells. On microscopic examination, these cells may or may not demonstrate mucin production. In cytology preparations, a typical adenocarcinoma presents as 3-dimensional or papillary clusters of neoplastic cells with nuclear pleomorphism (variability of cellular size and shape), increased nuclear to cytoplasmic ratio, eccentric to central nucleus, and moderate amount of pale vacuolated and wispy cytoplasm in a background that may have necrosis and mitotic figures. Squamous cell carcinoma typically appears as individual cells or cohesive flat sheets of polygonal tumor cells with well-defined cell borders, intercellular bridges, central hyperchromatic nuclei, dense and often keratinized cytoplasm. Bizarre cell shapes (tadpole cells) and abundant inflammatory necrotic debris are often present in squamous cell carcinomas.

Diagnostic Findings, Part III

The surgical core biopsy demonstrated an infiltrative tumor consisting of glandular elements with pleomorphic and hyperchromatic nuclei (Figure 1B) as compared to normal histology (Figure 1C). Increased mitotic activity was also noted. Immunohistochemical (IHC) findings showed that the tumor cells were positive for TTF1, Napsin-A, and negative for p40 (Figure 1D-F) consistent with the expected pattern for an adenocarcinoma. The specimen was also sent to the molecular genetics laboratory for mutation analysis.

Questions/Discussion Points, Part III

How Are Immunohistochemical Stains Used in Subclassifying Lung Tumors?

When morphological features suggest a malignancy, IHC studies may be required as the next step in the workup. In immunohistochemistry, antibodies to various cellular, nuclear, or structural proteins may provide evidence to the presence of specific cell types, lineages, or identify specific cancers. Immunohistochemical may even be performed on cytology preparations in which cellular material that is centrifuged and embedded in paraffin wax (known as a "Cell Block") may be used. In the evaluation of a possible lung cancer case, patterns of antibody staining to TTF-1, Napsin-A, CK5/6, and p40 usually prove to be helpful for typing of squamous cell carcinoma versus adenocarcinoma. Cells from a squamous cell carcinoma are generally CK5/6 and p40 positive and TTF-1 and Napsin A negative whereas adenocarcinoma cells are generally Napsin A and TTF-1 positive while being negative for p40 and CK5/6.[3]

Why Is Molecular Analysis Helpful in the Evaluation of Non-Small Cell Lung Cancer?

An increasing number of genetic aberrations have been identified in NSCLC. Many of these proteins are receptor kinases whose activities in normal cells are vital in cell proliferation, resistance to apoptosis, angiogenesis, and other important cellular activities.[4] However, mutations involving these genes could either cause them to become constitutively active or enable them to escape from their usual intracellular inhibitory mechanisms. Molecular identification of specific "driver" mutations is imperative for the selection of appropriate therapy as several targeted medications are now available to treat tumors with specific genomic variants.

Diagnostic Findings, Part IV

The molecular laboratory performed panel testing on the patient's tumor for a variety of known targetable mutations. The results identified a single DNA substitution in the epidermal growth factor receptor (*EGFR*) gene, which leads to the replacement of a Leucine at amino acid position 858 with an arginine (so termed L858R).

Questions/Discussion Points, Part IV

What Are Some Important Genomic Mutations in Non-Small Cell Lung Cancer?

Activating mutations in the *EGFR* (also called *ERBB1*) define a subset of patients with adenocarcinoma who are often nonsmokers, women, and/or of Asian ethnicity. Deletions in exon 19 and the point mutation of L858R constitute about 90% of all EGFR activating mutations. The mutations cause the autophosphorylation of the receptor, leading to uncontrolled downstream signaling and resistance to apoptosis and eventually

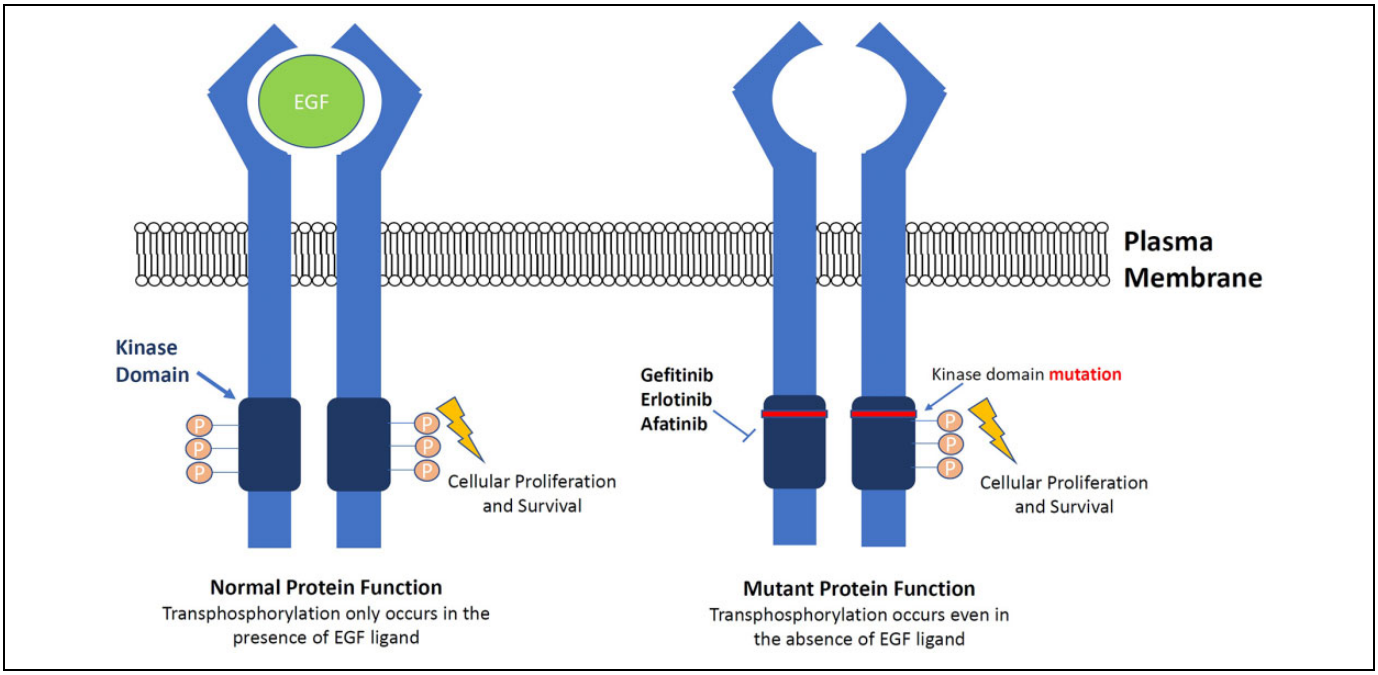

Figure 2. Graphical representation of the mechanism of the epidermal growth factor receptor. Under normal conditions, the downstream steps of the pathway are only activated when binding of the epidermal growth factor (EGF) ligand leads to phosphorylation of the kinase domains. With certain epidermal growth factor receptor (EGFR) mutations, cellular proliferation and survival occur even in the absence of the EGF ligand due to autocatalytic activity of the mutated kinases. Several drugs have been developed which inhibit the mutated kinases and improve clinical outcome.

tumorigenesis (Figure 2). Patients with these specific mutations are generally highly responsive to *EGFR* tyrosine kinase inhibitors (erlotinib, gefitinib, afatinib) and have a significantly better prognosis with targeted therapies than those without EGFR mutations.[5]

Another subset of patients with adenocarcinoma have been found to harbor rearrangements in the anaplastic lymphoma kinase (ALK) or ROS proto-oncogene 1 receptor tyrosine kinase (ROS1). These patients have been noted to present at a younger age and are frequently nonsmokers or former smokers. Both ALK and ROS1 are receptor tyrosine kinases which, when rearranged and fused with a partner gene, drive tumorigenesis in lung cancers. These patients are highly responsive to crizotinib, an inhibitor of the *ALK* kinase activity.[6]

BRAF, also referred to as *proto-oncogene B-Raf*, encodes a protein that is a member of the Raf kinase family of growth signal transduction protein kinases. *BRAF* mutations occur in around 3% of patients with lung adenocarcinoma. These patients are usually at a younger age and occur more frequently in patients who use tobacco or have a history of tobacco use. The most common mutation is a valine to glutamate substitution at codon 600 (V600E). Other mutations at codon 600 (V600K) and nearby codons in exon 15 (D594G) and mutations at codons 466 (G466 R) and 469 (G469A) in exon 11 have also been reported.[7] Current *BRAF* inhibitors such as vemurafenib and dabrafenib have shown some clinical activity. Various other agents targeting the *BRAF* pathway are currently being tested.[7]

KRAS encodes a GTPase downstream of *EGFR*. *KRAS* mutations are reported in 15% to 30% of lung adenocarcinomas. These patients are frequently current or former smokers. *KRAS* mutations and *EGFR* mutations are usually mutually exclusive. These mutations are most frequently found in codons 12 and 13 in exon 2. Therapies directed against mutated KRAS have not yet been proven clinically effective.[8]

The *RET proto-oncogene (RET)* and *MET proto-oncogene (MET)* encode receptor tyrosine kinases that have also been identified as driver mutations in a subset of lung cancers.[9,10] *RET* rearrangements are found in 1% to 2% of patients with NSCLC, usually younger patients with minimal smoking history. Therapies targeting the *RET* or *MET* pathway are now underway.

How Might We Identify Mutations in the Epidermal Growth Factor Receptor Gene?

In PCR-based *EGFR* mutation analysis, primers are designed to anneal to specific mutant or wild-type *EGFR* sequences. Using this "allele specific" PCR allows for great sensitivity; however, only those mutations for which primers are specifically designed will be detected. In recent years, the emergence of massively parallel sequencing techniques, known collectively as next-generation sequencing (NGS) methods, allows for high-throughput DNA sequencing of hundreds of DNA fragments simultaneously. Next-generation sequencing has the advantage of being able to detect all variants within the regions

of interest. Compared to PCR-based assays, NGS is more expensive and there is a considerable amount of work in both the technical and analytical portions of the testing, which leads to a longer turnaround time for resulting.

Diagnostic Findings, Part V

Given the extent of the patients disease and identification of the L858R sensitizing mutation, the patient was started on a tyrosine kinase inhibitor and subsequent imaging showed a marked tumor response to the treatment. Eighteen months later, the patient began to experience new symptoms of wheezing and shortness of breath. A follow-up chest X-ray revealed multiple additional nodules in both lung fields.

Questions/Discussion Points, Part V

What Is the Most Likely Explanation for the Patient's New Lung Findings?

The most likely explanation of the new lung nodules in a patient previously treated with an *EGFR* tyrosine kinase inhibitor is acquired resistance. This occurs when tumor cells develop new secondary mutations in the *EGFR* gene, which cause the receptor to overcome the blockade by the original targeted therapy. A point mutation leading to an amino acid change at position 790 (T790M) accounts for about one half of these cases of acquired resistance in lung adenocarcinomas.[11]

What Testing Methods Are Available to Confirm the Clinical Suspicion?

Up until recently, to confirm the presence of a secondary *EGFR* mutation, additional cellular material needed to be obtained. Today, there are developments that are leading to tests, which may allow for mutation testing in a less invasive manner. Rapidly growing tumor cells often shed fragments of DNA into the blood stream when they undergo necrosis or apoptosis. The DNA fragments are known as circulating tumor DNA (ctDNA). Detecting mutations within ctDNA may be used for therapeutic decision-making. Additionally, it is being investigated as an ancillary technique to monitor patients as it has been demonstrated that increasing levels of ctDNA correlate with extent and stage of the cancer.

What Are the Advantages and Disadvantages of the New Testing Method Mentioned Above?

Circulating tumor DNA samples are noninvasive compared to conventional FNAs and biopsies. Testing of ctDNA samples provides a rapid way to screen patients for possible mutations which can be treated with newer generations of anti-*EGFR* therapies.[12,13] Usually, only trace amounts of ctDNA are present in the plasma, and as such, the sensitivity of detecting mutations in the plasma may be significantly lower than when testing tissue directly. However, when the mutation is positively identified, it may be relied upon for initiation of third-generation tyrosine kinase inhibitors. One additional disadvantage of ctDNA is that whereas with tissue a pathologist can ensure the quality and tumor content of the submitted material, with ctDNA such preanalytic assessment is absent.

Teaching Points

- When evaluating a lung nodule suspicious for malignancy, several minimally invasive methods, including transbronchial FNA, BAL, EBUS-FNA, and percutaneous CT-guided FNA may be used to obtain cellular material for diagnosis.
- Approximately 95% of all lung cancers are classified as either SCLC or NSCLC. The subclassification of NSCLC can usually be determined by morphology and immunohistochemistry.
- Morphologically, lung adenocarcinoma usually presents as clusters of neoplastic cells with nuclear pleomorphism, increased nuclear to cytoplasmic ratio, eccentric to central nucleus, and moderate amount of pale vacuolated cytoplasm with or without the presence of intracellular mucin.
- Pathologists often utilize a variety of IHC antibodies to aid in the identification of neoplastic tissue. Lung adenocarcinoma cells are generally positive for Napsin A and TTF-1, while being negative for p40 and CK5/6.
- Determining *EGFR* mutation status in lung adenocarcinoma is important because targeted therapies are available for cancers harboring certain mutations.
- A number of other genetic aberrations involving *ALK, ROS1, BRAF, MET, ERBB2 [HER2], KRAS,* and *RET* have been identified in lung adenocarcinoma, some of which also have "companion" therapies.
- Secondary mutations in *EGFR* often develop after to initial targeted therapy, as tumor cells acquire additional mutations to evade the drugs inhibitory mechanisms.
- Novel testing methods such as NGS-based platforms and plasma ctDNA assays provide additional tools oncologists utilize to tailor therapy for their patients.

Declaration of Conflicting Interests

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