The Clinical Relevance of Pathologic Subtypes in Metastatic Lung Adenocarcinoma

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Introduction: The International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification of lung adenocarcinoma recommends identification of pathologic patterns in metastatic samples where possible. We investigated the clinical relevance of these patterns.

Methods: Patients with a surgical biopsy of lung adenocarcinoma from a metastatic site were included. Slides were reviewed by an anatomical pathologist identifying the histologic patterns of solid with mucin, acinar, micropapillary, papillary, and assigning a major adenocarcinoma subtype according to the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. *EGFR* and *KRAS* mutation testing were performed on formalin-fixed, paraffin-embedded blocks. Mutations were detected by high resolution melting assay with high resolution melting-positive samples confirmed by Sanger sequencing.

Results: One-hundred patients were included. The major histologic subtype prevalence was as follows: solid (50), acinar (29), micropapillary (20), and papillary (1). Of 100 patients, 45 received no systemic therapy with no overall survival differences seen by histologic subtype and 55 received systemic therapy (chemoradiotherapy with curative intent or palliative chemotherapy). Worse survival was seen in the major solid histologic subtype compared with major acinar (hazard ratio 0.32 [95% confidence interval 0.15–0.68], $p = 0.003$) and micropapillary subtypes (hazard ratio 0.34 [95% confidence interval, 0.17–0.69], $p = 0.003$). The major solid histologic subtype was less likely to harbor *EGFR* mutations ($p = 0.006$) and was less frequent in never smokers ($p = 0.010$) compared with other histologic subtypes.

Conclusion: The major solid histologic subtype of lung adenocarcinoma at metastatic sites is associated with shorter overall survival on systemic anticancer therapy. Furthermore, the major solid histologic subtype is less likely to harbor *EGFR* mutations. These results require validation in larger cohorts.

Key Words: Metastatic lung adenocarcinoma, Histopathology, EGFR, KRAS.

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The morphologic heterogeneity of lung adenocarcinoma has long been recognized. The 2004 World Health Organization (WHO) Classification of Lung Tumors, which is recommended for use in reporting of resection specimens only, identified several different morphologic subtypes of lung adenocarcinoma including acinar, papillary, and solid with mucin patterns and bronchioloalveolar carcinoma.[1] Furthermore, the 2004 WHO classification recommended that where more than one morphologic subtype was present in a tumor, it should be classified as adenocarcinoma of mixed subtype. Over time, it became clear that this diagnostic term, adenocarcinoma of mixed subtype, was of little clinical utility given that nearly 95% of tumors fell under this definition.[2]

In 2011, a new classification for resected lung adenocarcinoma was proposed by the International Association

for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and the European Respiratory Society (ERS).[3] The IASLC/ATS/ERS classification built on previous pathologic studies that demonstrated subtypes with favorable and unfavorable patient outcomes after surgical resection for early stage disease. Multiple independent groups have demonstrated the superiority of the IASLC/ATS/ERS classification over the WHO system in providing prognostic information in patients undergoing resection of early stage lung adenocarcinoma independent of pathologic stage,[4–11] with only one group failing to demonstrate an association.[12]

In addition, for the first time, the new IASLC/ATS/ERS classification provided a classification scheme for reporting of lung cancer in small biopsies and cytology specimens. As part of this proposal, it is suggested that when lung adenocarcinoma is diagnosed in a small biopsy/cytology specimen, the pathologist should "describe (the) identifiable patterns present." Given that there is minimal evidence for this recommendation, the authors of the new classification posed the research question: "In specimens from metastatic sites, is there any clinical significance to recognizing histologic patterns, including the predominant pattern?"[3]

Previously, the distinction between the different subtypes of non–small-cell lung carcinoma in metastatic disease had no bearing on treatment decisions. This has changed recently because of two major discoveries that have had a marked impact on clinical practice. First, patients with metastatic squamous cell carcinoma (SqCC) have been found to be at risk of significant toxicity from bevacizumab caused by major hemorrhage.[13] In addition, patients with metastatic squamous cell carcinoma had inferior survival outcomes, in comparison with those with adenocarcinoma and large cell carcinoma, when treated with pemetrexed-based chemotherapy caused by high expression of thymidylate synthase.[14,15] Second, the prevalence of oncogenic driver mutations, some associated with targeted therapies, differs between adenocarcinoma[16] and squamous cell carcinoma.[17] Review of the literature reveals that only one group has investigated the impact of subtyping of adenocarcinoma in specimens from patients with unresectable lung adenocarcinoma, classifying tumors according to the 1981 WHO classification (the contemporary classification at the time of their study). In this work, the presence of particular adenocarcinoma subtypes had no impact on overall survival (OS).[18,19]

In this study, we aimed to explore the clinical relevance of subtyping adenocarcinoma in biopsy specimens from metastatic sites in patients with metastatic lung adenocarcinoma, according to the new IASLC/ATS/ERS classification. We also examined the relationship of the adenocarcinoma subtypes to mutations in the epidermal growth factor receptor (*EGFR*) and *KRAS* genes.

MATERIALS AND METHODS

Patients

The Human Research and Ethics Committee at St. Vincent's Hospital approved this study. A review of two prospectively maintained clinical databases and associated tumor board meetings was conducted to identify patients who had had surgical sampling of lung adenocarcinoma from a metastatic site between 2000 and 2010. All patients had pathologically confirmed adenocarcinoma defined as a malignant epithelial tumor with histopathologic patterns including acinar, papillary, micropapillary, and solid with mucin adenocarcinoma, defined according to the new IASLC/ATS/ERS classification.[3] Patients included with stage III disease underwent mediastinal sampling for diagnosis and staging, but did not proceed to definitive surgical resection. All identified patients with stage IV lung adenocarcinoma underwent either surgical resection or sampling of a metastatic deposit. Clinical information was collected from the hospital medical records and records of treating medical oncologists and surgeons. The definition of a never smoker was a person with lifetime equivalent consumption of fewer than 100 cigarettes.

Histologic Evaluation

In patients who had undergone multiple surgical procedures for metastatic disease, the case containing the largest amount of tumor tissue was chosen to allow sufficient tissue for *EGFR* and *KRAS* analyses. The size (recorded as the largest dimension of a specimen in millimeters), location, and number of tumors were obtained from the pathology reports. A pathologist reviewed all hematoxylin and eosin (H&E) slides from each case. Patients were excluded at the time of pathologic review if the specimen was very small, if crush artifact was present precluding clear recognition of different adenocarcinoma patterns making it impossible to assign a major histologic pattern, or if there was insufficient available tissue for molecular testing.

The presence of different adenocarcinoma patterns, including acinar, papillary, micropapillary, and solid with mucin, as defined by the new IASLC/ATS/ERS classification, was recorded as a binary variable. It was possible in each case to identify a major histologic pattern; however, it was not possible to assign percentages to the different histologic patterns present. We chose to use the term "major" for the most prominent histologic pattern observed in metastatic samples. This term was used to avoid confusion with the recommendation of the new IASLC/ATS/ERS classification to use the term "predominant" for the most prominent histologic pattern identified in a resection specimen from the lung. All cases underwent immunostaining with TTF1 (clone SPT24, NovoCastra, Newcastle Upon Tyne, United Kingdom).

Molecular Pathology

Deparaffinization and DNA extraction

A formalin-fixed, paraffin-embedded block with adequate tumor for molecular analysis was chosen from each case, and a tumor-rich area was circled on the corresponding glass slide. This region was sampled from each block using two mm diameter dermatology core punches. The punched core tumor tissues were deparaffinized with 800 μl of xylene by incubating for seven minutes, followed by washings with 800 μl of 100 and 70% ethanol. After removal of 70% ethanol, tumor tissues were incubated at 55°C for 15 minutes for removal of residual ethanol. Genomic DNA was extracted using the

