Comprehensive Pathological Analyses in Lung Squamous Cell Carcinoma

Single Cell Invasion, Nuclear Diameter, and Tumor Budding Are Independent Prognostic Factors for Worse Outcomes

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Introduction: For lung squamous cell carcinomas, there are no pathological findings that have been universally accepted as prognostic factors, with the exception of pathological stage. Tumor budding and nuclear grade have been recognized as a poor prognostic factor in other carcinomas. In this study, we investigated whether pathological findings could determine prognosis in lung squamous cell carcinomas.
Methods: All available tumor slides from patients with surgically resected, solitary lung squamous cell carcinomas (1999–2009) were reviewed ($n = 485$; stage I/II/III, 281/136/68). Tumors were evaluated for differentiation, subtypes (keratinizing, nonkeratinizing, basaloid pattern, papillary growth, and clear cell feature), tumor nest size (tumor budding and single cell invasion), and nuclear grade (nuclear diameter and mitosis). Overall survival (OS) was estimated using the Kaplan-Meier method (stratified by pathological stage), and group differences were investigated using the stratified log-rank test and the Cox proportional hazards model.
Results: OS was significantly decreased in patients with versus without single cell invasion ($p = 0.002$ for the entire tumor and $p = 0.001$ for tumor edge), with large versus small nuclei ($p = 0.011$), and with high versus low grade tumor budding ($p < 0.001$ for maximum and $p = 0.007$ for total). In multivariate analyses, single cell invasion (hazard ratio [HR], 1.47–1.49), nuclear diameter (HR, 1.09–1.33), and tumor budding (HR, 1.04) were independent prognostic factors of OS. However, histologic subtyping including keratinizing, nonkeratinizing, basaloid, and clear cell subtypes did not show prognostic significance.
Conclusions: Pathological factors can help stratify prognosis in patients with lung squamous cell carcinomas.

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Currently, the tumor, node, metastasis (TNM) stage rather than any specific histologic feature is the most reliable prognostic predictor of non–small-cell lung cancers.[1] However, recently for lung adenocarcinoma, in addition to the TNM staging, the new international multidisciplinary histologic classification proposed by the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and European Respiratory Society (ERS) in 2011[2] has led to identification of the prognostic significance of the predominant histological patterns, and this has been validated in separate, large cohort studies (>400 patients) across multiple countries.[3–6] Traditionally, lung squamous cell carcinomas have been graded by the degree of keratinization (well, moderately, and poorly differentiated tumors). In the current World Health Organization (WHO) classification of lung carcinomas, squamous cell carcinomas are classified into papillary, clear cell, small cell, and basaloid subtypes; nevertheless, these have not been shown to have prognostic or other clinical significance.[7] Furthermore, no alternative histologic features or grading system have been identified that clinicians could use to predict patient clinical outcome.

Because histologic subtyping of lung squamous cell carcinoma has not proven to be associated with survival, we considered evaluating two approaches to assessment of patterns of tumor invasion: single cell invasion and tumor budding, which have been demonstrated to have prognostic significance in several types of cancers. One initial study identified single cell invasion as an unfavorable prognostic indicator in patients with lung squamous cell carcinomas.[8] Tumor budding is defined as the presence of isolated small tumor nests composed of less than five tumor cells in the stroma of the invasive tumor edge and it corresponds to significant tumor invasiveness.[9,10] It has been shown to correlate with an unfavorable clinical outcome (i.e., patient survival and disease recurrence) in colorectal cancer.[9,10] Interestingly enough, tumor budding may also exhibit

the process of epithelial mesenchymal transition, which regulates the epithelial tumor cells transformation into the mesenchymal phenotype, thus increasing the capacity of migration and invasion.[11–13] In addition to tumor budding, the size of the tumor nests (tumor clusters composed of ≤15 tumor cells) was determined to be a poor prognostic factor for the histological risk grading system of head and neck squamous cell carcinomas.[14,15] Despite the aforementioned correlations, comprehensive analyses on the prognostic value of tumor budding and tumor nest size have not been performed using a large cohort of resected lung squamous cell carcinomas.

A universally recognized histologic grading system for lung cancer has not been established. The clinical utility of using a nuclear grading system (e.g. mitotic count and nuclear atypia) has already been established in other major cancers such as breast carcinoma.[16,17] For lung adenocarcinoma, data are emerging for architectural and nuclear grading approaches that hopefully will lead to a uniform grading system in the near future.[18–20] After evaluating all of the nuclear features in stage I lung adenocarcinomas, our group has recently determined that a higher mitotic count is an independent predictor of a higher risk of recurrence. We then proposed a new grading system that combined architectural features (2011 IASLC/ATS/ERS classification) and nuclear grade (mitotic count).[21] However, for lung squamous cell carcinoma, a grading system for the prediction of patient's outcomes has not been rigorously investigated.

In this study of a large series of patients with resected lung squamous cell carcinomas, we performed comprehensive analyses of pathological factors (tumor differentiation, histologic subtype, tumor budding, tumor nest size, and nuclear grade). We investigated whether any of the pathological factors correlated with clinical outcomes (overall survival [OS] and disease recurrence), independent of pathological stage.

MATERIALS AND METHODS

Patients

This retrospective study was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center (MSKCC). We reviewed all patients with solitary lung squamous cell carcinoma who underwent surgical resection at MSKCC between 1999 and 2009; tumor slides were available for histologic evaluation from 485 of those patients. Clinical data were collected from the prospectively maintained Thoracic Surgery Service lung carcinoma database, and disease stage was assigned on the basis of the 7th edition of the American Joint Committee on Cancer TNM Staging Manual.[22] On chest computed tomography (CT), the tumor locations were divided into two categories: "peripheral lesion" when located within the outer third ellipse, and "nonperipheral lesion" when located within the middle third (intermediate lesion) or within the inner third (central lesion).[23,24]

Histologic Evaluation

All available hematoxylin and eosin (H&E) stained slides were reviewed by two pathologists (K.K. and W.D.T.) using an Olympus BX51 microscope (Olympus, Tokyo, Japan) with a standard 22-mm diameter eyepiece. Both pathologists had no knowledge of those patients' clinical outcomes.

Tumors were graded by a degree of squamous differentiation into well, moderately, and poorly differentiated, in accordance with the 2004 WHO classification of lung carcinomas.[7] In the well-differentiated tumors, there were tumor nests composed of differentiated keratinocyte-like tumor cells with prominent keratinization (layered and cytoplasmic keratin) and intercellular bridges. In the poorly differentiated tumors, squamous structure was only noticeable in a small area of the tumor. The moderately differentiated tumors showed an intermediate degree of squamous differentiation that was between well and poorly differentiated tumors.

Histologic subtyping was performed in a similar fashion to nasopharyngeal carcinomas in the 2005 WHO Classification, Pathology and Genetics of Head and Neck Tumours; they were classified as nonkeratinizing, keratinizing, and basaloid squamous cell carcinomas.[25] The percentage of keratinizing pattern, including layered (Fig. 1*A*) and cytoplasmic keratinization (Fig. 1*B*), was recorded, and then tumors were classified as having a keratinizing subtype when there was greater than or equal to 5% keratinizing pattern of the entire tumor whereas nonkeratinizing subtypes were defined as having less than 5% keratinizing pattern (Fig. 1*C*). The basaloid pattern was defined as tumor nests showing prominent peripheral palisading of tumor cells with scanty cytoplasm (high nuclear/cytoplasmic ratio) and a greater amount of hyperchromatic nuclei (Fig. 1*D*).[7] The percentage of basaloid pattern was recorded and then the tumors were classified as having a basaloid subtype if there was greater than 50% basaloid pattern as previously recommended.[26,27] The percentage of papillary growth was recorded in 5% increments. Clear

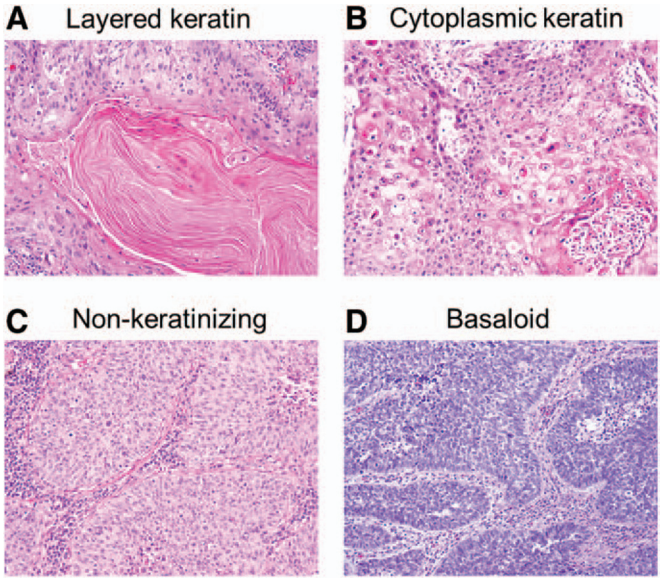

FIGURE 1. Histologic subtypes (hematoxylin and eosin-stain; original magnification, x200: *A–D*). *A*, keratinizing subtype with layered keratin. (*B*) Keratinizing subtype with cytoplasmic keratinization. (*C*) Nonkeratinizing subtype. (*D*) Basaloid subtype.

