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Validation of a Molecular and Pathological Model for Five-Year Mortality Risk in Patients with Early Stage Lung Adenocarcinoma

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Introduction: The aim of this study was to validate a molecular expression signature [cell cycle progression (CCP) score] that identifies patients with a higher risk of cancer-related death after surgical resection of early stage (I-II) lung adenocarcinoma in a large patient cohort and evaluate the effectiveness of combining CCP score and pathological stage for predicting lung cancer mortality.

Methods: Formalin-fixed paraffin-embedded surgical tumor samples from 650 patients diagnosed with stage I and II adenocarcinoma who underwent definitive surgical treatment without adjuvant chemotherapy were analyzed for 31 proliferation genes by quantitative real-time polymerase chain reaction. The prognostic discrimination of the expression score was assessed by Cox proportional hazards analysis using 5-year lung cancer-specific death as primary outcome.

Results: The CCP score was a significant predictor of lung cancer-specific mortality above clinical covariates [hazard ratio (HR) = 1.46 per interquartile range (95% confidence interval = 1.12–1.90; $p = 0.0050$)]. The prognostic score, a combination of CCP score and pathological stage, was a more significant indicator of lung cancer mortality risk than pathological stage in the full cohort (HR = 2.01; $p = 2.8 \times 10^{-11}$) and in stage I patients (HR = 1.67; $p = 0.00027$). Using the 85th percentile of the prognostic score as a threshold, there was a significant difference in lung cancer survival between low-risk and high-risk patient groups ($p = 3.8 \times 10^{-7}$).

Conclusions: This study validates the CCP score and the prognostic score as independent predictors of lung cancer death in patients with early stage lung adenocarcinoma treated with surgery alone. Patients with resected stage I lung adenocarcinoma and a high prognostic score may be candidates for adjuvant therapy to reduce cancer-related mortality.

Key Words: Carcinoma, Nonsmall cell lung cancer, Real-time polymerase chain reaction, Risk stratification.

(*J Thorac Oncol.* 2015;10: 67–73)

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Disclosure: Raphael Bueno has received an honoraria, research funding, and travel expenses from Myriad Genetics, Inc. Yifan Zheng and Michael A. Archer received institutional research funding from Myriad Genetics, Inc. Edward Kim is a consultant for and has received research funding from Myriad Genetics, Inc. Massimo Barberis is a consultant for GlaxoSmithKline and Roche. David J. Harrison is a consultant for Myriad Genetics, Inc. Elisha Hughes, Susanne Wagner, Alexander S. Gutin, PhD, Jerry S. Lanchbury, Joshua T. Jones, Kristen Rushton, Jennifer Saam, Richard J. Wenstrup, and Anne-Renee Hartman are employees of Myriad Genetics, Inc. and Myriad Genetic Laboratories, Inc. and receive salaries and stock. Corinne Gustafson, Ignacio Wistuba, and William A. Wallace have no conflicts to disclose. This research was supported by Myriad Genetics, Inc.

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DOI: 10.1097/JTO.0000000000000365.

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Lung cancer is one of the most common cancers in the United States and Europe and the leading cause of cancer death for both men and women in the United States, with approximately 160,000 patient deaths per year. Nonsmall cell lung cancer (NSCLC) comprises 85% of lung cancer cases of which up to 40% have adenocarcinoma histology. Patients diagnosed with stage I and II adenocarcinoma (localized disease) have 5-year overall mortality rates ranging from 30% in stage IA, 50% in stage IB disease, and up to 66% in stage II.[1,2]

The results of the Lung Adjuvant Cisplatin Evaluation meta-analysis established surgical resection followed by adjuvant chemotherapy with a cisplatin doublet as standard of care in patients with stage II and III disease.[3–5] However, this analysis failed to demonstrate a significant benefit for patients with stage IA and IB disease. A separate study, CALGB 9633, comprised of patients with stage IB disease, demonstrated a statistically significant survival advantage for patients with tumors ≥4 cm who received adjuvant therapy.[6] The significant mortality rate from lung cancer at 5 years for patients with stage I disease

despite complete surgical resection suggests that some patients may benefit from adjuvant chemotherapy although molecular markers to identify those patients are lacking.[1,7]

Defining the population at high risk of recurrence will allow for rational clinical trials that will determine the best therapies for these patients. There is a particularly acute need currently for developing such a strategy as the recent introduction of low-dose computed tomographic scans or other screening modalities for high-risk populations will lead to increased numbers of patients with early stage disease.[8] The ability to identify those patients with a high rate of recurrence, for whom adjuvant chemotherapy might provide benefit, is crucial in reducing the mortality from NSCLC. The fundamental role of this strategy is to identify which patients with early stage lung adenocarcinoma should be subjected to the risk of adjuvant therapy and which are unlikely to benefit from it.

Molecular signatures have been developed to assist in defining the risk of death with early stage disease.[9–12] However, a recent review highlighted the unsolved issues in the development and analysis of gene signatures in general, and lung cancer signatures in particular.[13] Very few of these signatures have been rigorously tested in combination with pathological variables, and even fewer have been applied to formalin-fixed clinical samples. Currently, no gene signatures have been included in clinical practice guidelines for the treatment of early stage resectable lung cancer.

We previously described the development and validation of an RNA expression signature based on cell cycle progression (CCP) genes to predict death from lung cancer.[14] In that analysis, the CCP score was a highly significant, independent predictor of cancer-specific mortality in adenocarcinomas in three independent datasets. Pathological stage remained an independent prognostic factor besides the CCP score, which prompted us to model a combined prognostic score of CCP and pathological stage based on the data in the CCP validation study. The combined score integrated molecular and clinical data to obtain a superior predictor of outcome than either variable alone.

The purpose of this study was to further validate the association of CCP with 5-year lung cancer mortality after adjusting for clinical parameters. We also sought to investigate the prognostic score as a predictor of 5-year lung cancer mortality risk and to establish a cut point for classifying patients into low- and high-risk groups. This study validates the CCP and prognostic score as robust molecular markers that predict death from early stage adenocarcinoma and provide useful information to determine which patients need to be considered for additional therapy to improve survival.

MATERIALS AND METHODS

Patients

Samples for this study were collected from consecutive population cohorts surgically treated at Brigham and Women's Hospital (BWH; Boston, MA) and the Royal Infirmary of Edinburgh (RIE; Edinburgh, United Kingdom) with appropriate Institutional Review Board approvals. Inclusion criteria were patients with NSCLC with adenocarcinoma histology, stage I-II disease according to 7th edition International Association for the Study of Lung Cancer (IASLC) guidelines, complete resection of the primary tumor, no treatment with radiation or chemotherapy before surgery, no adjuvant treatment with radiation or chemotherapy within 12 weeks of surgery, and at least 1-month of follow up. Patients diagnosed with previous lung cancer or synchronous lung cancers were excluded. Adenocarcinoma subtypes were assessed in the BWH cohort and are provided in Supplemental Table 1 (Supplemental Digital Content 1, http://links.lww.com/JTO/A710). To ascertain the primary outcome measure of death from lung cancer, clinical records or death records and ICD-10 codes were retrieved and reviewed. The secondary outcome measure was overall survival.

BWH provided 655 samples. Twelve samples were excluded based on little/no tumor or incorrect tumor pathology. The majority of samples (641/655) generated passing molecular scores but only 474 samples matched the above selection criteria, had complete clinical data and passing molecular scores. All samples were reviewed by a pulmonary pathologist to confirm diagnosis and tumor content before being sent for molecular analysis. Of 205 eligible samples from the RIE, 190 (92.6%) had passing molecular scores and 176 samples conformed to all inclusion criteria and had full clinical data. This study was conducted and reported according to REMARK guidelines.[15] All samples were rendered non-identifiable so that all laboratory analysis was blinded to any clinical or pathological data.

Expression Assay

All assay procedures were fully developed before initiation of the validation study and implemented in a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory. Experimental details of the CCP score have been described.[14] Briefly, tumor tissue was marked on a hematoxylin and eosin-stained section of formalin-fixed paraffin-embedded (FFPE) samples by a pathologist and two 5 to 10 μm sections of tumor tissues were excised by macro-dissection. Tissue was pooled and total RNA extracted with the FFPE miRNEasy Kit (Qiagen, Valencia, CA). RNA yield was determined using a Nanodrop 2000 Spectrophotometer (ThermoScientific, Waltham, MA) and RNA quality was assessed by the expression as described in the passing criteria below; 500 ng of total RNA were DNase-treated (Sigma-Aldrich, St. Louis, MO) and reverse transcribed with the High Capacity cDNA Archive Kit (Life Sciences, Foster City, CA). For pre-amplification, 60 ng of RNA-equivalent cDNA and a multiplex of all gene primers were setup in triplicate polymerase chain reaction (PCR) reactions. After 14 cycles, preamplified material was diluted 1:20 and used to inoculate custom Taqman Low Density arrays (Life Technologies, Grand Island, NY). Expression levels of 15 housekeeping genes and 31 cell cycle target genes were quantified as Ct values at a predefined threshold. Passing criteria for calculation of CCP scores included amplification of a minimum of 13 housekeeping genes and 22 cell cycle genes with measurable raw Ct values and a standard deviation of less than 0.5 between CCP scores from the three replicates for each sample. A list of genes that constitute the CCP score is provided in Supplemental Table 2 (Supplemental Digital Content 1, http://links.lww.com/JTO/A710).

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