ORIGINAL ARTICLE

# Unique Genetic and Survival Characteristics of Invasive Mucinous Adenocarcinoma of the Lung

*Hyo Sup Shim, MD, PhD,*† Mari-Kenudson, MD,* Zongli Zheng, PhD,* Matthew Liebers, BSc,* Yoon Jin Cha, MD,† Quan Hoang Ho, BSc,* Maristela Onozato, MD, PhD,* Long Phi Le, MD, PhD,* Rebecca S. Heist, MD, MPH,‡ and A. John Iafrate, PhD**

**Introduction:** Invasive mucinous adenocarcinoma is a unique histologic subtype of lung cancer, and our knowledge of its genetic and clinical characteristics is rapidly evolving. Here, we present next-generation sequencing analysis of nucleotide variant and fusion events along with clinical follow-up in a series of lung mucinous adenocarcinoma.

**Methods:** We collected 72 mucinous adenocarcinomas from the United States and Korea. All had been previously assessed for *KRAS* and *EGFR* mutations. For *KRAS* wild-type cases ($n$ = 30), we performed deep targeted next-generation sequencing for gene fusions and nucleotide variants and correlated survival and other clinical features.

**Results:** As expected, *KRAS* mutations were the most common alteration found (63% of cases); however, the distribution of nucleotide position alterations was more similar to that observed in gastrointestinal tumors than other lung tumors. Within the *KRAS*-negative cases, we found numerous potentially targetable gene fusions and mutations, including *CD74-NRG1*, *VAMP2-NRG1*, *TRIM4-BRAF*, *TPM3-NTRK1*, and *EML4-ALK* gene fusions and *ERBB2*, *BRAF*, and *PIK3CA* mutations. Unexpectedly, we found only two cases with *TP53* mutation, which is much lower than observed in lung adenocarcinomas in general. The overall mutation burden was low in histologically confirmed mucinous adenocarcinomas from the public The Cancer Genome Atlas exome data set, regardless of smoking history, suggesting a link between *TP53* status and mutation burden in mucinous tumors. There was no significant difference for recurrence-free survival between stage-matched mucinous and nonmucinous adenocarcinomas. It was notable that all recurrence sites were in the lungs for completely resected cases.

**Conclusions:** Our data suggest that mucinous adenocarcinoma is typified by (1) frequent *KRAS* mutations and a growing list of gene fusions, but rare *TP53* mutations, (2) a low mutation burden overall, and (3) a recurrence-free survival similar to stage-matched nonmucinous tumors, with recurrences limited to the lungs.

**Key Words:** Lung, Adenocarcinoma, Mucinous, Mutation, Gene fusion, Targeted therapy.

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Lung cancer is a leading cause of cancer-related mortality,[1] and adenocarcinoma is its most common histologic type.[2] Invasive mucinous adenocarcinoma, formerly known as mucinous bronchioloalveolar carcinoma, is a distinct variant of adenocarcinoma of the lung, accounting for approximately 5% of lung adenocarcinomas.[2] Its histology is unique among the primary lung cancers and is typified by a columnar or goblet cell structure with basally located nuclei and abundant intracytoplasmic mucin. Invasive mucinous adenocarcinoma is well known as having a distinct clinical presentation and genetic profile compared with nonmucinous adenocarcinoma.[2–6] Patients with invasive mucinous adenocarcinoma frequently present with a pneumonia-like pattern and with multifocal and multilobar lesions.[3] There are conflicting data about the relative prognosis of patients with mucinous adenocarcinoma.[7,8] In terms of genetic alterations, invasive mucinous adenocarcinoma shows a strong correlation with *KRAS* mutations.[4–6]

However, comprehensive molecular or clinical studies on invasive mucinous adenocarcinoma have been limited so far because the histology is relatively rare compared with other subtypes. Although a comprehensive molecular profiling of lung adenocarcinomas from The Cancer Genome Atlas (TCGA) has been published,[9] a detailed analysis of this subtype is warranted. Recently, *CD74-NRG1* fusions have been discovered in lung mucinous adenocarcinoma,[10–12] showing that these tumors are likely genetically unique.

To address the genetic and survival characteristics of invasive mucinous adenocarcinoma of the lung, we performed targeted next-generation sequencing for gene fusions and mutations, analyzed TCGA lung adenocarcinoma data, and investigated clinical features in this subtype when compared with nonmucinous adenocarcinomas.

*Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts; †Department of Pathology, Yonsei University College of Medicine, Seoul, Korea; and ‡Department of Thoracic Oncology, Massachusetts General Hospital Cancer Center, Boston, Massachusetts.

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Address for correspondence: Department of Pathology, Massachusetts General Hospital, 55 Fruit Street, Jackson 1015A, Boston, MA 02114. E-mail: aiafrate@partners.org

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## MATERIALS AND METHODS

### Study Population

We collected a total of 83 mucinous adenocarcinomas from Massachusetts General Hospital, Boston, Massachusetts ($n$ = 35) and Yonsei University Severance Hospital, Seoul, Korea ($n$ = 48) (Supplementary Fig. 1, Supplemental Digital Content, http://links.lww.com/JTO/A842) under Institutional Review Board approval. All samples were resected specimens and formalin-fixed and paraffin-embedded. Two pulmonary pathologists (H.S.S. and M.M.-K.) reviewed the slides and confirmed the diagnosis based on the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classification.[2] All clinical information, such as age, gender, smoking status, and stage, were obtained from medical records. We also collected a total of 269 nonmucinous adenocarcinomas from Massachusetts General Hospital ($n$ = 63) and Yonsei University Severance Hospital ($n$ = 206) as a control group for survival analysis. All patients underwent surgical resection for curative intent. TCGA data for lung adenocarcinomas ($n$ = 230) were obtained from the public on-line cBioPortal database.[9,13,14] Histologic review for each case was done using Digital Slide Archive in the cBioPortal.

### SNaPshot Genotyping

Multiplexed targeted genotyping was done using the SNaPshot method as previously described.[15,16] The SNaPshot platform from Applied Biosystems (Life Technologies/Applied Biosystems, Foster City, CA) consisted of multiplexed polymerase chain reaction (PCR) and single-base extension reactions that generate fluorescent labeled probes designed to interrogate hot-spot mutation sites. The SNaPshot products were then resolved and analyzed using capillary electrophoresis.

### Sanger Sequencing for *EGFR* and *KRAS* Mutation

A representative formalin-fixed, paraffin-embedded block containing at least 50% viable tumor was selected for each sample. After proteinase K digestion, DNA was extracted using a DNeasy DNA isolation kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. Direct DNA sequencing of exons 18 through 21 of the *EGFR* gene and codons 12 and 13 of the *KRAS* gene was performed as previously described.[17,18]

### Anchored Multiplex PCR and Next-Generation Sequencing

To detect gene fusions and mutations, we used a gene enrichment method, anchored multiplex PCR, to perform next-generation sequencing using MiSeq (Illumina, San Diego, CA) platform as previously described in detail.[19] Total nucleic acid containing total RNA and genomic DNA were extracted from formalin-fixed paraffin-embedded tissue, using the Agencourt FormaPure Kit (Beckman Coulter, Indianapolis, IN). We used at least 50 ng of total nucleic acid for fusion analysis and 200 ng of genomic DNA for mutation analysis. The genes covered in each primer panel are shown in Supplementary Table 1 (Supplemental Digital Content, http://links.lww.com/JTO/A842).

### Immunohistochemisty

Formalin-fixed and paraffin-embedded tissues were sectioned with a thickness of 4 μm and stained with antibody for p53 (mouse monoclonal, clone DO-7, ready-to-use, Leica Biosystems, United Kingdom) using Leica Bond 6 automated stainer according to the manufacturer's protocol. p53 immunohistochemistry was considered to be abnormal when the expression was present in 50% or greater of the tumor cells or was completely negative.

### Statistical Analysis

Relationships between clinicopathologic parameters were evaluated using the chi-square test. Student's $t$ test was used to compare means between two independent groups. We planned a survival comparison study of mucinous cases and nonmucinous cases (controls) with four controls per case. Prior data indicated that the overall 5-year survival rate among controls is 60%. If the survival rate among mucinous cases is 80%, we needed to study at least 52 patients with mucinous adenocarcinoma and 208 control patients to be able to reject the null hypothesis that the survival rates for case and controls are equal with probability (power) 0.8. This sample size was calculated by Power and Sample Size Calculations (Version 3.1.2; Vanderbilt University, Nashville, TN). The disease-free survival and overall survival were evaluated using the Kaplan–Meier method, and statistical differences in survival times were determined using the log-rank test. Data analysis was conducted using SPSS v.17 (SPSS, Chicago, IL) or Prism 6 (GraphPad Software, San Diego, CA). Significance was defined as $p$ value less than 0.05.

## RESULTS

We identified 83 cases of mucinous adenocarcinoma, including 81 invasive mucinous adenocarcinomas, one mucinous adenocarcinoma in situ, and one minimally invasive mucinous adenocarcinoma (all defined using the IASLC/ATS/ERS criteria) and 269 nonmucinous cases from the case records of the Massachusetts General Hospital and the Yonsei University Severance Hospital (detailed information according to the institutions in Supplementary Table 2, Supplemental Digital Content, http://links.lww.com/JTO/A842). There were no significant differences between patients with mucinous adenocarcinoma and those with nonmucinous adenocarcinoma with respect to age, sex, smoking status, or stage (Table 1). Of 83 patients with mucinous adenocarcinoma, *KRAS* genotyping was previously done for 72 cases. For *KRAS* wild-type cases ($n$ = 30), we performed targeted deep sequencing for gene fusions and mutations using the anchored multiplex PCR method. We found driver mutations and fusions in 16 of the 30 cases (Fig. 1), including three cases with *KRAS* mutation not found with the prior less-sensitive methods (Supplementary Tables 3 and 4, Supplemental Digital Content, http://links.lww.com/JTO/A842). There was no significant difference between *KRAS* mutated ($n$ = 45) and *KRAS* wild-type ($n$ = 27) groups in terms of age, gender, smoking status, and stage (Table 2).

We identified gene fusions in nine cases, including four with a *CD74-NRG1* fusion, two with an *EML4-ALK* fusion, one each with a *VAMP2-NRG1* fusion, a *TRIM4-BRAF* fusion, and a *TPM3-NTRK1* fusion. We confirmed novel fusions using

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