Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Association for Academic Surgery

Common driver mutations and smoking history affect tumor mutation burden in lung adenocarcinoma

Masayuki Nagahashi, MD, PhD,[a,*] *Seijiro Sato, MD, PhD,*[b] *Kizuki Yuza, MD, PhD,*[a] *Yoshifumi Shimada, MD, PhD,*[a] *Hiroshi Ichikawa, MD, PhD,*[a] *Satoshi Watanabe, MD, PhD,*[c] *Kazuki Takada, MD, PhD,*[d] *Tatsuro Okamoto, MD, PhD,*[d,e] *Shujiro Okuda, PhD,*[f] *Stephen Lyle, MD, PhD,*[g] *Kazuaki Takabe, MD, PhD, FACS,*[a,h,i,j,k] *Masanori Tsuchida, MD, PhD,*[b] *and Toshifumi Wakai, MD, PhD, FACS*[a]

[a] *Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan*

[b] *Division of Thoracic and Cardiovascular Surgery, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan*

[c] *Department of Respiratory Medicine and Infectious Disease, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan*

[d] *Department of Surgery and Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan*

[e] *Department of Thoracic and Breast Surgery, Oita University Faculty of Medicine, Oita, Japan*

[f] *Division of Bioinformatics, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan*

[g] *University of Massachusetts Medical School, Worcester, Massachusetts*

[h] *Department of Surgical Oncology, Breast Surgery, Roswell Park Cancer Comprehensive Cancer Center, Buffalo, New York*

[i] *Department of Surgery, University at Buffalo Jacobs School of Medicine and Biosciences, The State University of New York, Buffalo, New York*

[j] *Department of Breast Surgery and Oncology, Tokyo Medical University, Tokyo, Japan*

[k] *Department of Surgery, Yokohama City University, Yokohama, Japan*

ARTICLE INFO

Article history:
Received 3 March 2018
Received in revised form
19 May 2018
Accepted 2 July 2018
Available online xxx

ABSTRACT

Background: Recent progress in genomic analysis using next-generation sequencing technology has enabled the comprehensive detection of mutations and tumor mutation burden (TMB) in patients. A high TMB (TMB-H) tumor is defined as one with high somatic mutational rates, which correlates with clinical responses to certain treatments such as immunotherapies. We determined TMB in lung adenocarcinoma and clarified the

This article was presented at 13th Annual Academic Surgical Congress held at Jacksonville, FL, on January 30-February 1, 2018.

* *Corresponding author*. Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Chuo-ku, Niigata City 951-8510, Japan. Tel.: +81 25 227 2228; fax: +81 25 227 0779.

E-mail address: mnagahashi@med.niigata-u.ac.jp (M. Nagahashi).

0022-4804/$ – see front matter

https://doi.org/10.1016/j.jss.2018.07.007

Keywords:
Next-generation sequencing
Tumor mutation burden
Lung adenocarcinoma
Immune checkpoint inhibitors
Anti-PD-1

characteristics of patients with TMB-H in relation to common driver mutations and smoking history.

Materials and methods: Genomic aberrations and TMB were determined in Japanese patients with lung adenocarcinoma ($n = 100$) using next-generation sequencing of 415 known cancer genes. TMB-H was defined as > 20 mutations per megabase (Mb) of sequenced DNA.

Results: The median TMB was 13.5 (5-33) mutations/Mb. Ten of 100 (10%) patients showed TMB-H, and the others showed low TMB (TMB-L). Only two of 10 (20%) patients with TMB-H had one of the common driver mutations (ALK and ERBB2 mutation), whereas 57 of 90 (63%) patients with TMB-L had one of the driver mutations, including ALK, EGFR, ERBB2, ROS, RET, and MET ($P < 0.05$). Notably, no EGFR mutation was observed in patients with TMB-H. Eight of 10 (80%) patients with TMB-H had recent smoking history, whereas only 17 of 90 (19%) patients with TMB-L had recent smoking history ($P < 0.001$).

Conclusions: We found that TMB-H is associated with smoking history, whereas TMB-L is associated with the common driver mutations in lung adenocarcinoma, which may impact treatment strategies for these patients.

Introduction

Lung cancer is the leading cause of cancer death worldwide.[1,2] Adenocarcinoma is the most common histologic type, which accounts for approximately 40% of lung cancers.[3] Lung adenocarcinoma usually arises in the distal airway and frequently occurs in current or former smokers.[4] However, interestingly, adenocarcinoma is also the most frequent cancer among lung cancers in nonsmokers.[5] The proportion of lifelong nonsmokers in lung cancer patients is reported to be high in Asia, and the population is thought to be genetically predisposed to lung cancer.[6] More recently it has been found that lung cancer in never-smoker Asian females is driven by oncogenic mutations such as EGFR.[7]

Immune checkpoint inhibitors, such as the anti-PD-1 therapy developed by Dr Honjo,[8,9] show dramatic effects on several types of solid tumors, including lung cancer.[10,11] Although the expectation of immune checkpoint inhibitors is tremendously high based on its dramatic efficacy, only a limited number of patients respond.[12] Furthermore, there is no definite biomarker for identifying which tumors respond to this therapy.[12]

Recent progress in genomic analysis using the next-generation sequencing (NGS) technology has enabled the comprehensive detection of mutations and the definition of tumor mutation burden (TMB) in cancer patients.[13-15] A high TMB (TMB-H) tumor is defined as one with high somatic mutational rates, which correlates with the generation of neoantigens and also to potential clinical responses to immunotherapies.[13] Interestingly, it has been reported that there is an association between smoking history and TMB-H in non-small cell lung cancer.[12]

Although sequencing studies of lung adenocarcinoma have been reported,[12,16] there is a paucity of data elucidating the relationship between comprehensive genomic data and clinical histories, such as smoking. We hypothesized that TMB-H is associated with smoking history in some patients with lung adenocarcinoma. The aim of this study was to identify TMB in patients with lung adenocarcinoma utilizing NGS and to seek association with smoking history and with common oncogenic driver mutations in lung adenocarcinoma.

Materials and methods

Patients

We analyzed a total of 100 patients diagnosed with stage I–IV lung adenocarcinoma according to American Joint Committee on Cancer (AJCC) Cancer Staging Manual seventh edition,[17] who had received an operation at Niigata University Medical and Dental Hospital from January 2008 to December 2014 or at Kyushu University from October 2013 to August 2015. Patients who satisfied following criteria were selected to be analyzed: a tumor content of >20% based on pathological examination of primary tumor with hematoxylin and eosin staining, radiological confirmation of lung adenocarcinoma with a consolidation/tumor ratio (C/T ratio) > 0.5 using thin-section computed tomography, and successful extraction of ≥150 ng DNA from the tumor sample. This study was approved by the Institutional Review Boards of Niigata University and Kyusyu University Hospital, and written informed consent was obtained from all patients.

Sequencing library preparation

We analyzed archival tissues of primary cancer from surgical specimens that were confirmed to be of high quality in the form of formalin-fixed, paraffin-embedded (FFPE) slides.[18] An independent pathologist evaluated the tumor content on hematoxylin and eosin stained slides for each study sample to ensure that > 20% tumor content was present. Where applicable, unstained slides were macrodissected to enrich for tumor content, and genomic DNA was extracted using the BiOstic FFPE Tissue DNA Isolation Kit (Mo Bio Laboratories, Inc; Carlsbad, CA). DNA quality of the samples was confirmed by QC ratio as previously described.[18] In brief, the quality of an adequate quantity of DNA was assessed by determining the Q-ratio, in which 41 bp and 129 bp targets were amplified by quantitative polymerase chain reaction and compared using a KAPA Human Genomic DNA Quantification and QC Kit (KAPA Biosystems). The 41 bp assay was used for absolute quantification of DNA samples. DNA with a Q-ratio (129 bp/41 bp) > 0.1 was designated as being of high enough quality for NGS