Rationale and Design of the Japan Molecular Epidemiology for Lung Cancer Study

Tomoya Kawaguchi,[1] Masahiko Ando,[2] Norimasa Ito,[3] Shun-Ichi Isa,[4] Akihiro Tamiya,[1] Shigeki Shimizu,[5] Hideo Saka,[6] Akihito Kubo,[7] Yasuhiro Koh,[8] Akihide Matsumura[3]

Abstract

We present the rationale for the Japan Molecular Epidemiology for Lung Cancer study designed to elucidate molecular mechanisms of carcinogenesis in smokers and never-smokers with non–small-cell lung cancer. This prospective, ongoing, multicenter study is being conducted nationwide in Japan. Although there is no doubt that active smoking is the major cause of lung cancer, the contribution of other possible factors, including environmental tobacco or wood smoke, human papilloma virus, radon, occupational exposures, and genetic susceptibility, is highly likely, based on studies of never-smokers with non–small-cell lung cancer. Because of the predominance of women in the never-smoker subgroup, the role of female hormones in lung cancer development has also been considered. We hypothesize that driver mutations, which are critical for the development of lung cancer, are triggered by the environmental factors with or without the influence of the hormone. The SWOG-led intergroup molecular epidemiology study S0424 was conducted to focus on these issues by using a detailed questionnaire and specimen collection in statistically significant cohorts of smokers and never-smokers from both sexes. The Japan Molecular Epidemiology for Lung Cancer study follows and extends the S0424 molecular epidemiology concept in principle by using a similar approach that will facilitate future comparisons between the studies but with a greater focus on more recently defined driver mutations and broad genomic sequencing.

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Introduction

Lung cancer is a leading cause of cancer-related morbidity and mortality in the world. Although the disease is predominantly caused by tobacco smoke, approximately 25% of all lung cancers worldwide are not attributable to this etiology. In fact, approximately 30% of Japanese patients with non–small-cell lung cancer (NSCLC) are never-smokers, as observed in a study that consisted of more than 20,000 patients.[1] Lung cancer in never-smokers differs significantly from that of smokers in clinical characteristics and in the distribution of oncogenic abnormalities, and it has been suggested to be a distinct disease.[2]

Although several possible explanations have been proposed, the cause of lung cancer in never-smokers remains unclear. Explanations include environmental tobacco smoke (ETS) exposure,[3] radon,[4] wood smoke,[5] occupational exposure,[6] oncogenic virus,[7,8] genetic change,[9] and sex hormone.[10,11] A Japan Public Health Center–based prospective study showed that, in Japan, second-hand smoke exposure is clearly related to the development of lung adenocarcinoma in never-smokers.[3] The study identified a statistically significant dose-response relationship between the quantity and the intensity of husbands' smoking and their wives' incidence of lung cancer. Our previous study with a detailed questionnaire in a prospective way enhances this finding that the development of epidermal growth factor receptor (*EGFR*) mutations is significantly associated with the dose of ETS exposure in never-smokers.[12] However, there are con-

[1]Department of Internal Medicine, National Hospital Organization, Kinki-chuo Chest Medical Center, Osaka, Japan
[2]Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan
[3]Department of Surgery, National Hospital Organization, Kinki-chuo Chest Medical Center, Osaka, Japan
[4]Clinical Research Center, National Hospital Organization, Kinki-chuo Chest Medical Center, Osaka, Japan
[5]Department of Pathology, National Hospital Organization, Kinki-chuo Chest Medical Center, Osaka, Japan
[6]Department of Respiratory Medicine, National Hospital Organization, Nagoya Medical Center, Nagoya, Japan
[7]Division of Respiratory Medicine and Allergology, Aichi Medical University School of Medicine, Nagakute, Japan
[8]Division of Drug Discovery and Development, Shizuoka Cancer Center Research Institute, Sunto-gun, Japan

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Address for correspondence: Tomoya Kawaguchi, MD, Department of Internal Medicine, National Hospital, Organization Kinki-chuo Chest Medical Center, Sakai, Osaka 591–8555, Japan
E-mail contact: t-kawaguchi@kch.hosp.go.jp

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flicting data published on the relationship between ETS and *EGFR* mutations in never-smokers with NSCLC. A study from Korea showed opposite results, in which the development of the mutation was inversely proportional to the ETS[13]; although, in the United States, there was no association between them.[14] A study with a well-designed and standardized questionnaire in a larger sample size is required to conclude this issue.

An oncogenic role for the HPV has been widely investigated in NSCLC.[7] Although all the published reports were retrospective analyses with potentially significant limitations and bias, the systematic review nevertheless suggested that the development of lung cancer in Asia can be attributed to some extent to HPV. Moreover, a different detection rate was observed geographically even within east Asia, with a higher rate in the southern area than in the northern regions. There is a substantial need to confirm these findings by using a standardized HPV detection methodology in a prospective study in Japanese patients.

The association between sex and lung cancer carcinogenesis is also an important consideration. Although studies provide conflicting results on the strength of this association, it has been postulated that women are more vulnerable to tobacco smoke–associated carcinogens than men. The large SWOG study S0424 was originally designed to address this issue by using a detailed questionnaire and NSCLC tissue specimens from smoker and never-smoker men and women with newly diagnosed stage I, stage II, or stage III NSCLC,[15] in which polycyclic aromatic hydrocarbons and aromatic amines of DNA adducts are measured to quantitate levels of DNA damage stratified by sex and the smoking status. Cigarette smoke contains a large number of carcinogens, and polycyclic aromatic hydrocarbons and aromatic amines are among the most important contributors to the carcinogenic process. The Japan Molecular Epidemiology for lung cancer (JME) study follows and extends the concept of S0424 by using a similar approach that will allow for direct comparison of data in the future.

Sex hormones, including estrogen and progesterone, have been suggested to play an important role in lung carcinogenesis. Results of epidemiologic studies showed that women were predominant in number in the never-smoking subpopulation. Further, results of large randomized studies suggest that estrogen plus progestin therapy is associated with an increased risk of lung cancer. The prospective Vitamins and Lifestyle Study followed a cohort of more than 36,000 peri- and postmenopausal women during 6 years of follow-up.[16] After adjusting for smoking and other confounding factors, the incidence of lung cancer was increased for those who used estrogen plus progestin. The risk was proportional to the duration of hormone exposure (hazard ratio 1.48 [95% CI, 1.03-2.12] for those with $\geq$10 years of exposure to estrogen plus progestin).

In terms of biologic function, estrogen receptors (ER) are expressed in diverse normal and neoplastic tissues, and mediate growth and maturation of normal tissue. A number of studies have noted expression of ERs in a large portion of lung tumors. In a couple of studies, the development of *EGFR* mutations was significantly associated with expression of ER β in NSCLC surgical specimens.[10,11] There have been no studies that systematically evaluated ER expression in lung cancer and its relationship with genetic mutations or environmental and reproductive risk factors.

Identification of driver mutations in NSCLC has been instrumental in improving treatment strategies. *ALK* (anaplastic lymphoma kinase) gene translocations have been demonstrated to be critical targets and biomarkers for crizotinib efficacy,[17] similar to *EGFR* mutations for gefitinib and erlotinib, and the discovery of other mutations for treatment is ongoing. The Lung Cancer Mutational Consortium in the United States[18] and the Lungscape project in the European Union[19] are currently exploring new molecular targets for treatment in lung cancer. Powerful tools for genome-wide characterization have been developed, including next-generation sequencing, which enables comprehensive examination of somatic mutations associated with carcinogenesis. The Cancer Genome Atlas is an ongoing global project that uses this technology to distill essential driver abnormalities from the background noise.[20] A focus of the JME study is to explore new driver mutations by using advanced technologies and approaches now available with regard to sex of the patient and tobacco smoke exposure. The association between oncogenic abnormality profiles and drug sensitivity and prognosis will also be examined.

In addition, the JME study is designed to investigate the relationship between ethnicity and NSCLC carcinogenesis. It is clear that NSCLCs are different in tumor biology between Caucasian and Asian patients. Gandara et al[21] showed that there was a significant difference in survival and toxicities between the US and the Japanese patients treated with carboplatin and paclitaxel in a "common arm" trial, in which the study design, eligibility criteria, and staging were similar. The median overall survival in the metastatic disease was 12 and 14 months for Japanese patients vs. 9 months for US patients ($P = .0006$).[21] As for *EGFR* mutations, the frequencies appear to be highly distinct; the high detection rate in Asia was reported consistently across publications. Different influences of smoking status on the development of NSCLC also was observed between the United States and Japan in population-based prospective studies. In a comparison of the Japanese cohort with US Cancer Prevention Study II during the same period,[22] Japanese never-smokers had an increased risk of lung cancer, whereas Japanese current smokers were at a lower risk of the cancer compared with those in the United States. To elucidate the mechanistic contributions of ethnic differences, there is a need to collaborate in comprehensive and global approaches for examining development of NSCLC as well as the clinical behavior and outcome.

Objectives

The primary objective of this study is to assess surgical lung specimens from patients with stage I, stage II, stage IIIA, or stage IIIB NSCLC for driver mutations, expression of HER2 and ER α and ER β, the presence of smoking-associated DNA adducts, and evidence of HPV, and to explore new molecular markers by using next-generation sequencing. By using information collected before surgery on patient demographics, smoking history and occupational exposures, carcinogenic mechanisms will be elucidated in never-smokers and ever-smokers. Secondary objectives are to examine whether the relapse rate, disease-free survival, and overall survival time differ among the patients with different mutational