SYMPOSIUM PRESENTATION

Risk Analysis of Environmental Chemicals on Lung Carcinogenesis

Katsumi Imaida, Masanao Yokohira, Nozomi Hashimoto, Toshiya Kuno

Abstract

Lung cancer is one of the most common cancers in the world, and the incidence of lung cancer is increasing. Risk analysis of environmental chemicals on lung carcinogenesis is particularly important. Detection of chemopreventive agents of lung carcinogenesis is also important to reduce our risk of lung cancer. For that purpose, it is necessary to establish reliable in vivo animal models of lung carcinogenesis. The A/J mouse is a mouse strain sensitive to lung carcinogens, and also develops spontaneous lung tumors without any chemical treatment. We have demonstrated that a treatment of 4-(methylnitrosamino)-1-(3-pyridyle)-1-butanone (NNK), a tobacco specific nitrosamine, or 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (MeIQx), a heterocyclic amine, induced lung tumors in the female A/J mouse in 16 and 32 weeks. The lung tumors developed in the A/J mouse are histopathologically classified as adenocarcinomas, adenomas, and alveolar cell hyperplasias. Some of these types of lung cancer are similar to those of human lung cancer. We also investigated the chemopreventive effects of bovine LF (bLF) on different phases of NNK-induced lung tumorigenesis in A/J mice. The A/J mouse is very useful mouse strain as a reliable in vivo model, which can be used for risk analysis of lung carcinogenesis.

Key Words: Risk analysis - lung carcinogenesis - environmental chemicals - chemoprevention - mouse

Asian Pacific J Cancer Prev, **11**, 9-12

Introduction

Lung cancer is one of the main causes of death due to cancer in the United States, Japan and most of other countries. In order to identify hazardous compounds or risk factors in our environment, it is very important to use suitable animal experimental bioassay models. However, in terms of lung carcinogenesis bioassay models, there are no good animal models for predicting lung carcinogens, and promoting or chemopreventive compounds. Therefore, it is important to detect carcinogenic or tumor promoting substances of lung carcinogenesis in our environment, and the detection of chemopreventive agents of lung carcinogenesis is also important to reduce our risk of lung cancer. It is necessary to establish reliable in vivo animal models of lung carcinogenesis for that purpose.

Environmental Factors for Lung Carcinogenesis

Many dietary factors have been reported to modify carcinogenesis in different organs of man and experimental animals(Sugimura, 2000). With regard to lung carcinogenesis, only a few promoters have been identified, such as bleomycin, butylated hydroxytoluene, glycerol or iron administration.

We investigated the influence of dietary high fat diet on 4-nitroquinoline 1-oxide (4NQO)-induced lung tumorigenesis in the ICR mouse (Imaida et al., 1989). A total of 160, 6-week-old male ICR mice (Charles River Japan Inc., Atsugi) were divided into 4 equal groups: Groups 1 and 2 were given a single injection of 4NQO subcutaneously at a dose of 15 mg/kg body weight, and Groups 3 and 4 received a single injection of 10 ml/kg body weight of the oil mixture without 4NQO. One week later, Groups 1 and 3 were placed on a diet (CRF-1, Oriental Yeast Co. Ltd., Tokyo: 3.5kcal/g) containing 20% corn oil (high fat diet; 4.7 kcal/g), the main components of the CRF-1 diet and the corn oil being as follows: oleic acid (22.4: 32.8%), linolic acid (50.2: 51.9%), linoleic acid (4.6: 1.8%). Groups 2 and 4 were maintained on basal diet without supplement. Ten mice from each group were sacrificed at weeks 15 and 18, and all surviving animals (19 to 20 mice per group) were sacrificed at week 25. The incidences of lung lesions in 4NQO-Fat group were always higher than in 4NQO alone group, and although the differences were not significant at each time point, Peto's trend test revealed a significant difference between the two groups overall. Furthermore, the mean numbers of lung tumors/animal were significantly different between 4NQO-Fat and 4NQO alone groups 1 and 2 sacrificed at weeks 15 and 25. This study demonstrated that dietary high

Onco-Pathology, Department of Pathology and Host-Defenses, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan
E-mail: imaida@med.kagawa-u.ac.jp

fat clearly enhances the development of 4NQO-initiated mouse lung tumors (Imaida et al., 1989). Similar results were also published for benzo[a]pyrene-induced hamster respiratory tumor development and N-nitrosobis(2-oxopropyl)amine-induced hamster lung tumorigenesis. Other dietary factors of lung carcinogenesis were reported for heterocyclic amines, mutagenic compounds found in cooked foods, especially 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (MeIQx) (Sinha et al., 2000).

Tobacco-smoking has been epidemiologically associated with lung cancer in humans. Experimentally, however, animal models of tobacco-smoking induced lung cancer have not been successful. An A/J mouse model that is responsive to cigarette smoke has been described by Witschi et al.(2005) Lung adenocarcinomas in mouse, including the A/J mouse strain, are morphologically similar to human lung adenocarcinomas, and furthermore, many of the signaling pathways with genetic and epigenetic alterations in oncogenes and tumor suppressor genes are identical to those that are found in human lung cancer. Molecular aspects of lung cancer in humans and mice have also been well analyzed. K-ras mutations in lung cancer are well documented. These findings show that the mouse lung carcinogenesis model is very suitable tool for identifying human lung carcinogens, promoters or modifiers, and chemopreventive agents of lung carcinogenesis.

Metabolism and Lung Cancer

Cigarette smoke has been epidemiologically associated with lung cancer in humans. Kamataki et al. (2005) reported that Japanese male smokers with CYP2A6 gene deletion-type polymorphism were shown to have a reduced lung cancer risk in a hospital-based case control study. Furthermore, CYP2A6 gene deletion reduced oral cancer risk in betel quid chewers in Sri Lanka (Topcu et al., 2002). 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), one of tobacco-specific N-nitrosamines, plays an important role in tobacco-related human lung cancer, and it has strong potential to induce lung tumorigenesis in rodents. If one of the causes of human lung cancer is dependent on metabolic activation of a tobacco-specific nitrosamine, inhibition of CYP2A6 by chemicals may result in chemoprevention of tobacco-related lung cancer. Therefore, we examined the potential inhibitory effects of 8-methoxypsorarene (8-MOP), a potent human CYP2A6 inhibitor, on NNK-induced lung tumorigenesis in female A/J mice.

Pretreatment of 8-MOP significantly reduced tumor incidence from 93.8% to 16.7% (50 mg/kg body weight) and 20.0% (12.5 mg/kg body weight), and tumor multiplicity from 5.97 to 0.23 (50 mg/kg body weight) and 0.25 (12.5 mg/kg body weight) tumors/mouse. These results indicate that 8-MOP, a potent human CYP2A6 inhibitor, is a strong chemopreventive agent for NNK-induced A/J mouse lung tumorigenesis (Takeuchi et al., 2003).

Dose dependent inhibitory effects of dietary 8-MOP on NNK-induced lung tumorigenesis were also examined. mRNA levels of CYP2A4 and CYP2A5 were also analyzed

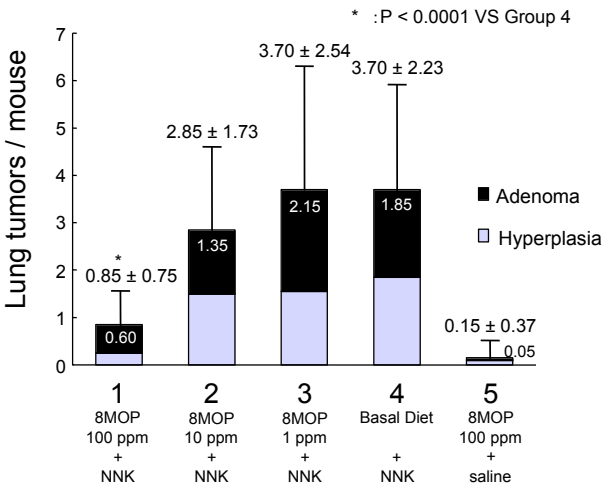

Figure 1. Multiplicities of Microscopic Lung Nodules

using the reverse transcriptase-polymerase chain reaction. Mouse CYP2A4 and CYP2A5 differ from each other by only 11 amino acids, and these enzymes are closely related to human CYP2A6. The results showed that the values of tumor incidences and tumor multiplicities in the 100 ppm 8-MOP treated group were significantly lower than in the NNK-treated alone group. 8-MOP reduced the multiplicities of NNK-induced lung proliferative lesions in a dose dependent manner (see Figure 1). The relative quantifications of CYP2A4 and CYP2A5 mRNAs in livers and lungs of A/J mice were not influenced by the 8-MOP treatment (Takeuchi et al., 2006). The mechanisms and timing of the 8-MOP chemoprevention of NNK-induced mouse lung tumorigenesis have also been reported (Miyazaki et al., 2005). Pretreatment of mice with 8-MOP inhibited the incidence and multiplicities of macroscopically and microscopically examined lung lesions. However, treatment of 8-MOP on days 1, 3 and 7 after NNK administration did not affect the incidence and multiplicities of the observed lung lesions. These results suggest that 8-MOP abolished NNK-induced lung tumorigenesis via the inhibition of an initiation event in lung carcinogenesis, but not subsequent events including promotion of carcinogenesis. In this experiment, the expression of mRNA for CYP2A5, but not for CYP2A4 or CYP2A12, in mouse lung was proved by reverse transcriptase-polymerase chain reaction, probably indicating that CYP2A5 present in the mouse lung was involved in the metabolic activation of NNK. Interestingly, tumor cells in lung adenoma were positive for CYP2A immunohistochemistry (see Figure 2).

Analysis of Chemopreventive Agents

Since lung cancer is one of the most common causes of mortality and morbidity in the world, new therapeutic strategies such as chemoprevention are a high priority. Lactoferrin (LF) is a multifunctional, iron-binding glycoprotein present mainly in external secretions, such as breast milk, tears, saliva and seminal fluid, and in the secondary granules of neutrophils. It was originally identified as a mucosal host defense mediator and anti-inflammatory modulator. Furthermore, LF has shown

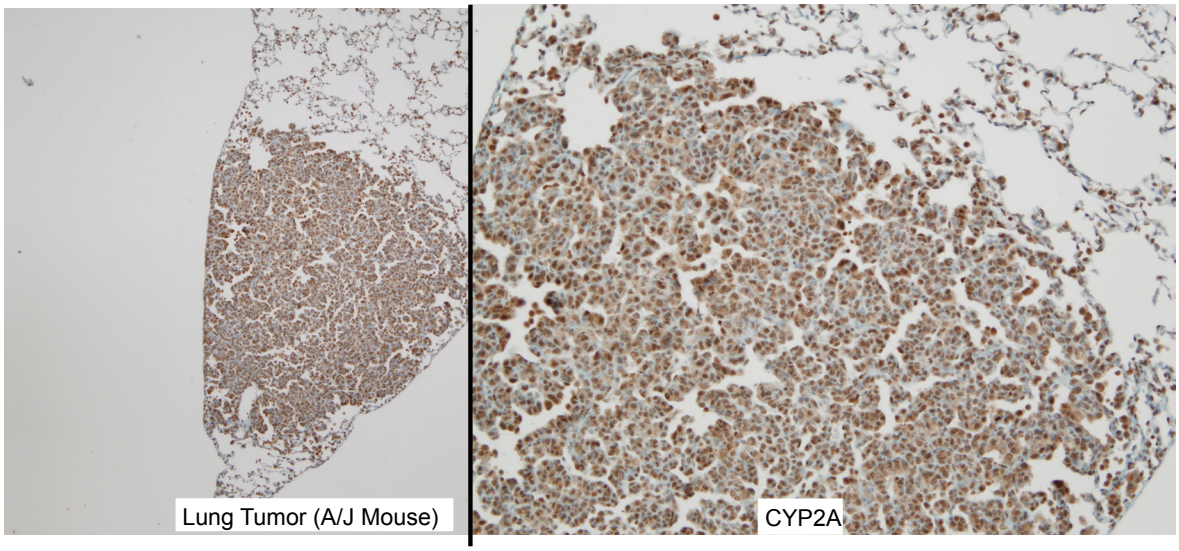

Figure 2. Immunohistochemical detection of CYP2A in Lung Tumors

anti-tumor effects in vitro and in vivo in the colon, tongue, esophagus, urinary bladder, and possibly in the lung(Tsuda et al., 2000). We investigated the effects of orally administered LF using a mouse model with NNK-induced lung tumors, which histopathologically resemble human lung adenocarcinomas (Estensen et al., 2004). For the longer bLF treatment, macroscopically counted lung nodules were significantly reduced by orally administered 2% bLF during the post-initiation phase. There were no significant differences in incidences of microscopically diagnosed lung proliferative nodules (Matsuda et al., 2006). Immunohistochemical data of PCNA, a cell proliferation marker, and cleaved caspase-3, an apoptosis marker, were analyzed. The mean PCNA-labeling index ratios in hyperplasias and adenomas of bLF during the post-initiation phase were lower than those of the NNK alone group. The mean cleaved caspase-3-labeling index ratios in adenomas showed a tendency to increase in the bLF treated groups compared to the NNK alone group, but without statistical significance. These data showed chemopreventive effects of dietary bLF on NNK-induced lung tumorigenesis in mice, through modification of cell proliferation and/or apoptosis (Matsuda et al., 2006).

Conclusion

Risk analysis of environmental chemicals is very important in order to reduce potentially harmful chemicals in our environment. Therefore, detection of chemopreventive agents of lung carcinogenesis is important for reducing the risk of lung cancer. The A/J mouse is sensitive to lung carcinogens and spontaneous lung tumors also develop without any chemical treatment. NNK, a tobacco specific nitrosamine, and MeIQx, a heterocyclic amine, induced lung tumors in 16 and 32 weeks in female A/J mice. The lung tumors developed in the A/J mouse were histopathologically classified as adenocarcinomas, adenomas, and alveolar cell hyperplasias. Using the A/J mouse, we reported that 8-MOP, a potent human CYP2A6 inhibitor, is a strong chemopreventive agent of NNK-induced of lung tumorigenesis. We also investigated the effects of bLF on different phases of NNK-induced lung tumorigenesis in A/J mice. bLF administered during the post-initiation phase caused a significant reduction in macroscopical lung nodules. bLF might inhibit NNK-induced mouse lung tumorigenesis, only when given in the post-initiation phase, through modification of cell proliferation and/or apoptosis.

The A/J mouse is very useful mouse strain as a reliable in vivo model of lung carcinogenesis, which can be used for determining chemopreventive agents of lung carcinogenesis.

References

Estensen RD, Jordan MM, Wiedmann TS, et al (2004). Effect of chemopreventive agents on separate stages of progression of benzo[alpha]pyrene induced lung tumors in A/J mice. *Carcinogenesis*, **25**, 197-201.

Imaida K, Sato H, Okamiya H, Takahashi M, Hayashi Y (1989). Enhancing effect of high fat diet on 4-nitroquinoline 1-oxide-induced pulmonary tumorigenesis in ICR male mice. *Jpn J Cancer Res*, **80**, 499-502.

Kamataki T, Fujieda M, Kiyotani K, Iwano S, Kunitoh H (2005). Genetic polymorphism of CYP2A6 as one of the potential determinants of tobacco-related cancer risk. *Biochem Biophys Res Commun*, **338**, 306-10.

Matsuda Y, Saoo K, Hosokawa K, et al (2006). Post-initiation chemopreventive effects of dietary bovine lactoferrin on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in female A/J mice. *Cancer Lett*, **246**, 41-6.

Miyazaki M, Yamazaki H, Takeuchi H, et al (2005). Mechanisms of chemopreventive effects of 8-methoxypsoralen against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced mouse lung adenomas. *Carcinogenesis*, **26**, 1947-55.

Sinha R, Kulldorff M, Swanson CA, et al (2000). Dietary heterocyclic amines and the risk of lung cancer among Missouri women. *Cancer Res*, **60**, 3753-6.

Sugimura T (2000). Nutrition and dietary carcinogens. *Carcinogenesis*, **21**, 387-95.

Takeuchi H, Saoo K, Matsuda Y, et al (2006). Dose dependent inhibitory effects of dietary 8-methoxypsoralen on NNK-induced lung tumorigenesis in female A/J mice. *Cancer*

Lett, **234**, 232-8.
Takeuchi H, Saoo K, Yokohira M, et al (2003). Pretreatment with 8-methoxypsoralen, a potent human CYP2A6 inhibitor, strongly inhibits lung tumorigenesis induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in female A/J mice. *Cancer Res*, **63**, 7581-3.
Topcu Z, Chiba I, Fujieda M, et al (2002). CYP2A6 gene deletion reduces oral cancer risk in betel quid chewers in Sri Lanka. *Carcinogenesis*, **23**, 595-8.
Tsuda H, Sekine K, Takasuka N, Toriyama-Baba H, Iigo M (2000). Prevention of colon carcinogenesis and carcinoma metastasis by orally administered bovine lactoferrin in animals. *Biofactors*, **12**, 83-88.
Witschi H (2005). A/J mouse as a model for lung tumorigenesis caused by tobacco smoke: strengths and weaknesses. *Exp Lung Res*, **31**, 3-18.