Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 338 (2005) 306–310
BBRC
www.elsevier.com/locate/ybbrc

Review

Genetic polymorphism of *CYP2A6* as one of the potential determinants of tobacco-related cancer risk

Tetsuya Kamataki [a,*], Masaki Fujieda [a], Kazuma Kiyotani [a], Shunsuke Iwano [a], Hideo Kunitoh [b]

[a] *Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan*
[b] *National Cancer Center Hospital, Tokyo 104-0045, Japan*

Received 26 August 2005
Available online 15 September 2005

Abstract

Analyzing the *CYP2A6* gene of subjects who showed a poor metabolic phenotype toward SM-12502, we discovered a novel mutant allele (*CYP2A6*4C*) lacking the whole *CYP2A6* gene. Using genetically engineered *Salmonella typhimurium* expressing a human CYP, we found that CYP2A6 was involved in the metabolic activation of a variety of nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) contained in tobacco smoke. Taking these results into consideration, we hypothesized that the subjects carrying the *CYP2A6*4C* allele had lower risk of tobacco-related lung cancer. In accordance with our hypothesis, our epidemiological studies indicated that smokers homozygous for the *CYP2A6*4C* allele showed much lower odds ratios toward cancer risk. Other mutant alleles reducing the CYP2A6 activity, besides *CYP2A6*4C*, also reduced the risk of lung cancer in smokers, particularly of squamous-cell carcinoma and small-cell carcinoma, both smoking-related cancers. 8-Methoxypsoralen, an inhibitor of CYP2A6, efficiently prevented the occurrence of adenoma caused by NNK in A/J mice.

Keywords: P450 2A6; *CYP2A6*4C*; Single nucleotide polymorphism(s); NNK; Smokers; Smoking; Case-control study

Roles of cytochrome P450 (CYP) in carcinogenesis

CYP is now widely known as a family of enzymes metabolizing a wide variety of xenobiotics, including drugs and carcinogens. Most carcinogens are metabolically activated to generate ultimate carcinogens, leading to chemical carcinogenesis. Such functions of the monooxygenase in activating chemical carcinogens must not have been assumed 50 yr ago, when oxygenases were discovered. Thus, the discovery of monooxygenases contributes greatly to the mechanism of chemical carcinogenesis. CYP1A1 is a well-studied enzyme in carcinogenesis for its capacity to activate polycyclic aromatic hydrocarbons, including benzo[*a*]pyrene. CYP1A2 activates heterocyclic amines such as 2-amino-3,5-dimethly-imidazo[4,5-*f*]quinoline [1]. CYP2A6 and CYP2E1 are responsible for the metabolic activation of *N*-nitrosamines [1].

Another important aspect of the xenobiotic metabolizing enzyme is the existence of genetic polymorphisms. The genetic polymorphism of *CYP* causes interindividual differences in the undesired as well as therapeutic effects of drugs. Also, it has been postulated that the genetic polymorphisms cause interindividual differences in the susceptibility of individuals to carcinogens.

Genetic polymorphism of *CYP* and cancer risk

It has been generally accepted that most cancers are caused by environmental chemicals contained in foods and tobacco smoke [2,3]. These environmental chemicals are first metabolically activated by enzymes present in the body to generate reactive intermediates, which subsequently bind covalently to DNA. Almost all the damaged DNA

* Corresponding author. Fax: +81 11 706 4978.
E-mail address: SNC78123@nifty.com (T. Kamataki).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.08.268

is repaired by repairing enzymes, while some of the injured DNA remains unrepaired, which is called cancer initiation. Thus, the metabolic activation process can be regarded as the first step by which environmental chemicals cause cancer. However, no direct evidence has been reported to date to confirm this idea in humans.

CYP plays central roles in the activation of many environmental chemicals to generate reactive intermediates [4]. Thus, it can be expected that the activity of this enzyme determines the risk of cancer. Accordingly, the idea that the genetic polymorphism of *CYP* affects cancer risk has been demonstrated. The first report on the association between genetic polymorphism of *CYP* and lung cancer risk appeared on CYP2D6 in 1984 [5]. However, many conflicting results have been reported; the idea is still a matter of controversy [6]. Afterward, the effects of the genetic polymorphism of *CYP1A1* on lung cancer risk were proposed by Kawajiri and his associates [7]. This idea seemed to be reasonable, since Kellermann et al. [8] reported that the inducibility of arylhydrocarbon hydroxylase in lymphocytes closely correlated with lung cancer risk. However, this idea has not been reproduced in other ethnic groups, probably because of low frequency of the *Msp* I site mutation of the *CYP1A1* gene [9,10].

Discovery of whole gene deletion-type polymorphism (*CYP2A6*4C*)

Among many forms of cytochrome P450, CYP2A6 is known to be responsible for the metabolism of drugs including coumarin [11,12], nicotine [13], tegafur [14,15], fadrozole [16], methoxyflurane [17], and valproic acid [18]. The genetic polymorphism of *CYP2A6* was first suggested by evidence that there was large interindividual variation in the capacity for coumarin 7-hydroxylation [19,20].

During the course of the development of an anti-platelet activating agent, (+)-*cis*-3,5-dimethyl-2-(3-pyridyl)thiazoline-4-one hydrochloride (SM-12502), in the Sumitomo Pharmaceutical Industry, three volunteer subjects showed a poor metabolic phenotype toward this agent. Following their clinical data, we determined which enzyme(s) was involved in the metabolism of SM-12502, and found that this agent was metabolized mainly by CYP2A6 [21]. Analyzing the *CYP2A6* gene of the three subjects, we discovered a novel deletion-type polymorphism (*CYP2A6*4C*) [22,23]. We developed a gene diagnosis method for the *CYP2A6*4C* variant [24].

Establishment of *Salmonella typhimurium* tester strains carrying human CYP to predict the mutagenicity of chemicals in humans

Promutagens and carcinogens exert their mutagenicity and carcinogenicity after undergoing metabolic activation by enzymes, including CYP. Remarkable species differences are seen in both the activity and the amounts of corresponding forms of CYP. These species differences are recognized as one of the causes of species differences in responses to drugs and toxicants. In classical mutation assay systems to predict the mutagenicity of chemicals, the 9000*g* supernatant (S9) fraction of rat liver homogenates has routinely been used to activate promutagens in the *S. typhimurium* mutation assay. Because of species differences enzymes such as CYP, the S9 fraction prepared from human liver homogenates is sometimes used. However, there is still a disadvantage of this assay: the sensitivity is not high enough because of the possible trapping of reactive intermediates by components present on the surfaces of cell membranes. To overcome the disadvantages, we established new genetically engineered tester strains of *S. typhimurium* expressing human CYP together with NADPH-CYP reductase inside of the cells [25]. Using the established *S. typhimurium* strains, we examined many chemicals to evaluate the usefulness of the genetically engineered bacteria. When we tested *N*-nitrosamines to determine which human CYP enzyme was responsible for the mutagenic activation, we found that CYP2A6 was mainly involved in the *N*-nitrosamines with relatively bulky alkyl moieties, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), contained in tobacco smoke, while CYP2E1 was involved in the *N*-nitrosamines with relatively small alkyl groups, such as *N*-nitrosodimethylamine [26,27].

Large-scale epidemiological study on the association of genetic polymorphism of *CYP2A6* and tobacco-related cancer risk

Based on results obtained as mentioned above, we hypothesized that smokers carrying the *CYP2A6*4C* allele might have less risk of tobacco-related cancers, since the subjects lacking the *CYP2A6* gene cannot activate *N*-nitrosamines such as NNK and *N*′-nitrosonornicotine, which are contained in tobacco smoke as nicotine-derived carcinogens. The results of our epidemiological studies clearly showed that subjects homozygous for *CYP2A6*4C* have considerably less risk of lung cancer [28]. This tendency was seen in smokers but not in nonsmokers [29].

Further epidemiological studies to confirm the linkage between *CYP2A6* genetic polymorphism and tobacco-related cancer risk

The aim of our next study was to confirm the possibility that the reduced lung cancer risk seen in subjects carrying the *CYP2A6*4C* allele was caused by the low metabolic capacity of CYP2A6, but not by a possible gene linkage between the *CYP2A6*4C* mutation and the mutation of oncogenes or anti-oncogenes.

After we discovered the *CYP2A6*4C* variant allele, our and other groups discovered many novel variants of the *CYP2A6* gene, such as *CYP2A6*7* [30], *CYP2A6*9* [31,32], and *CYP2A6*10* [33], which have been proven to decrease the catalytic capacity or expression level of

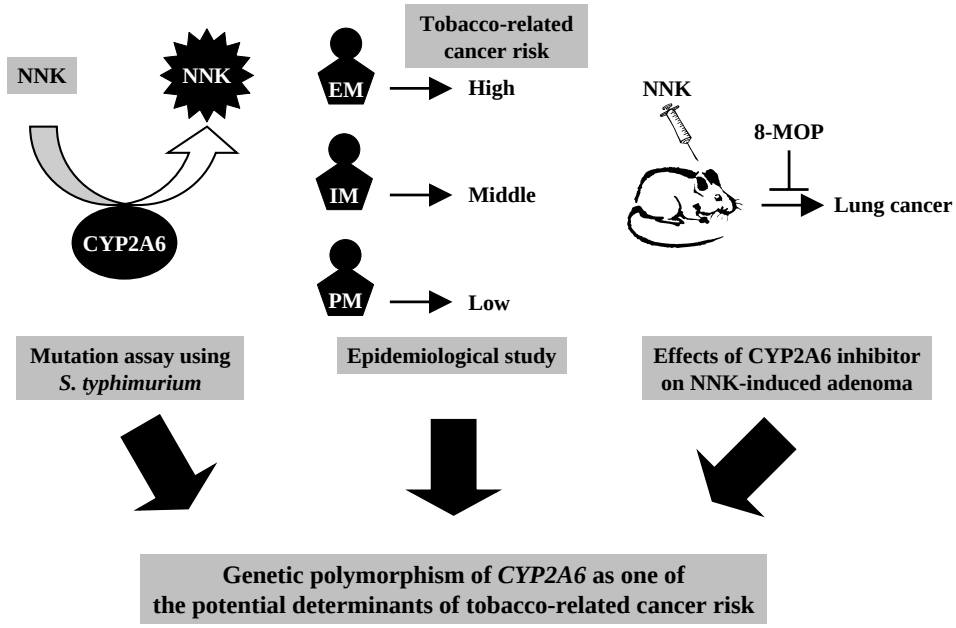

Fig. 1. Genetic polymorphism of *CYP2A6* is one of the determinants of tobacco-related cancer risk. NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; 8-MOP, 8-methoxypsoralen.

CYP2A6. If our hypothesis that the capacity of CYP2A6 to activate the *N*-nitrosamines is one of the key determinants of tobacco-related cancer risk is correct, tobacco-related cancer risk can be expected to decrease in association with these variant alleles. We analyzed our data again by classifying the subjects according to the histological types of lung cancer. Squamous-cell carcinoma and small-cell carcinoma are known to be the most frequent types of lung cancer in smokers. At least two papers have appeared demonstrating that adenocarcinoma is also caused by smoking of filter-equipped cigarettes [34,35]. We obtained clear epidemiological data indicating that the cancer risk was decreased by the predicted capacity (or genotypes) of CYP2A6 [36]. The tendency was seen more clearly in subjects suffering from squamous-cell carcinoma and small-cell carcinoma and less clearly in patients suffering from adenocarcinoma. These data were in complete agreement with our expectations. We also analyzed the same data after classifying subjects into two groups, heavy smokers and light smokers, smoking larger and smaller amounts of cigarettes than average (approx 38 pack-years), respectively. The reduced risk for lung cancer was seen more clearly in the heavy smokers than in the light smokers, lending support to the previous data that the genetic polymorphism of *CYP2A6* affects the lung cancer risk in smokers but not in nonsmokers. Together with the result that CYP2A6 was involved in the metabolism of nicotine [13], less consumption of cigarettes is also a factor in reduced risk of lung cancer. However, even when we calculated the data adjusting for the number of cigarettes, the reduced risk of lung cancer in subjects homozygous for *CYP2A6*4C* allele was clear.

Effects of 8-methoxypsoralen on NNK-induced adenoma in mice

Based on the results of epidemiology, it seemed possible to assume that the inhibitors of CYP2A6 reduced the risk of cancer. Instead of performing human prospective studies, we performed experiments using the A/J strain of mice. Pretreatment of mice with 8-methoxypsoralen, a known inhibitor of CYP2A6, completely prevented the occurrence of adenoma and hyperplasia caused by NNK, which supported our human epidemiological studies [37].

Conclusions

Genetic polymorphism of *CYP2A6* affected cancer risk in smokers but not in nonsmokers. The effects were in accordance with the capacity of CYP2A6 in subjects, as proven by the fact that other mutated alleles of the *CYP2A6* gene known to reduce the metabolic capacity also reduced the cancer risk. This hypothesis was supported experimentally. Pretreatment of mice with 8-methoxypsoralen clearly prevented the occurrence of adenoma caused by NNK. Based on these lines of evidence, it is suggested that genetic polymorphism of *CYP2A6* is one of the determinants of tobacco-related cancer risk (Fig. 1).

References

[1] F.P. Guengerich, T. Shimada, Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes, Chem. Res. Toxicol. 4 (1991) 391–407.

[2] R. Doll, R. Peto, The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today, J. Natl. Cancer Inst. 66 (1981) 1191–1308.
[3] M. Kee, Cancer causation in booklet form, Nature 303 (1983) 648.
[4] F.P. Guengerich, Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity, Chem. Res. Toxicol. 14 (2001) 611–650.
[5] R. Ayesh, J.R. Idle, J.C. Ritchie, M.J. Crothers, M.R. Hetzel, Metabolic oxidation phenotypes as markers for susceptibility to lung cancer, Nature 312 (1984) 169–170.
[6] N. Caporaso, M.R. DeBaun, N. Rothman, Lung cancer and *CYP2D6* (the debrisoquine polymorphism): sources of heterogeneity in the proposed association, Pharmacogenetics 5 (1995) S129–S134.
[7] K. Kawajiri, K. Nakachi, K. Imai, A. Yoshii, N. Shinoda, J. Watanabe, Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome *P450IA1* gene, FEBS Lett. 263 (1990) 131–133.
[8] G. Kellermann, C.R. Shaw, M. Luyten-Kellerman, Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma, N. Engl. J. Med. 289 (1973) 934–937.
[9] N. Drakoulis, I. Cascorbi, J. Brockmoller, C.R. Gross, I. Roots, Polymorphisms in the human *CYP1A1* gene as susceptibility factors for lung cancer: exon-7 mutation (4889 A to G), and a T to C mutation in the 3'-flanking region, Clin. Invest. 72 (1994) 240–248.
[10] I. Cascorbi, J. Brockmoller, I. Roots, A C4887A polymorphism in exon 7 of human *CYP1A1*: population frequency, mutation linkages, and impact on lung cancer susceptibility, Cancer Res. 56 (1996) 4965–4969.
[11] J.S. Miles, A.W. McLaren, L.M. Forrester, M.J. Glancey, M.A. Lang, C.R. Wolf, Identification of the human liver cytochrome *P*-450 responsible for coumarin 7-hydroxylase activity, Biochem. J. 267 (1990) 365–371.
[12] C.H. Yun, T. Shimada, F.P. Guengerich, Purification and characterization of human liver microsomal cytochrome P-450 2A6, Mol. Pharmacol. 40 (1991) 679–685.
[13] M. Nakajima, T. Yamamoto, K. Nunoya, T. Yokoi, K. Nagashima, K. Inoue, Y. Funae, N. Shimada, T. Kamataki, Y. Kuroiwa, Role of human cytochrome P4502A6 in *C*-oxidation of nicotine, Drug Metab. Dispos. 24 (1996) 1212–1217.
[14] K. Ikeda, K. Yoshisue, E. Matsushima, S. Nagayama, K. Kobayashi, C.A. Tyson, K. Chiba, Y. Kawaguchi, Bioactivation of tegafur to 5-fluorouracil is catalyzed by cytochrome P-450 2A6 in human liver microsomes *in vitro*, Clin. Cancer Res. 6 (2000) 4409–4415.
[15] T. Komatsu, H. Yamazaki, N. Shimada, M. Nakajima, T. Yokoi, Roles of cytochromes P450 1A2, 2A6, and 2C8 in 5-fluorouracil formation from tegafur, an anticancer prodrug, in human liver microsomes, Drug Metab. Dispos. 28 (2000) 1457–1463.
[16] O. Pelkonen, A. Rautio, H. Raunio, M. Pasanen, CYP2A6: a human coumarin 7-hydroxylase, Toxicology 144 (2000) 139–147.
[17] E.D. Kharasch, D.C. Hankins, K.E. Thummel, Human kidney methoxyflurane and sevoflurane metabolism. Intrarenal fluoride production as a possible mechanism of methoxyflurane nephrotoxicity, Anesthesiology 82 (1995) 689–699.
[18] A.J. Sadeque, M.B. Fisher, K.R. Korzekwa, F.J. Gonzalez, A.E. Rettie, Human CYP2C9 and CYP2A6 mediate formation of the hepatotoxin 4-ene-valproic acid, J. Pharmacol. Exp. Ther. 283 (1997) 698–703.
[19] S. Cholerton, M.E. Idle, A. Vas, F.J. Gonzalez, J.R. Idle, Comparison of a novel thin-layer chromatographic-fluorescence detection method with a spectrofluorometric method for the determination of 7-hydroxycoumarin in human urine, J. Chromatogr. 575 (1992) 325–330.
[20] A. Rautio, H. Kraul, A. Kojo, E. Salmela, O. Pelkonen, Interindividual variability of coumarin 7-hydroxylation in healthy volunteers, Pharmacogenetics 2 (1992) 227–233.
[21] K. Nunoya, Y. Yokoi, K. Kimura, T. Kodama, M. Funayama, K. Inoue, K. Nagashima, Y. Funae, N. Shimada, C. Green, T. Kamataki, (+)- *cis*-3,5-dimethyl-2-(3-pyridyl) thiazolidin-4-one hydrochloride (SM-12502) as a novel substrate for cytochrome P450 2A6 in human liver microsomes, J. Pharmacol. Exp. Ther. 277 (1996) 768–774.
[22] K.I. Nunoya, T. Yokoi, K. Kimura, T. Kainuma, K. Satoh, M. Kinoshita, T. Kamataki, A new *CYP2A6* gene deletion responsible for the in vivo polymorphic metabolism of (+)-*cis*-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride in humans, J. Pharmacol. Exp. Ther. 289 (1999) 437–442.
[23] K. Nunoya, T. Yokoi, Y. Takahashi, K. Kimura, M. Kinoshita, T. Kamataki, Homologous unequal cross-over within the human *CYP2A* gene cluster as a mechanism for the deletion of the entire *CYP2A6* gene associated with the poor metabolizer phenotype, J. Biochem. (Tokyo) 126 (1999) 402–407.
[24] N. Ariyoshi, Y. Takahashi, M. Miyamoto, Y. Umetsu, S. Daigo, T. Tateishi, S. Kobayashi, Y. Mizorogi, M.A. Loriot, I. Stucker, P. Beaune, M. Kinoshita, T. Kamataki, Structural characterization of a new variant of the *CYP2A6* gene (*CYP2A6*1B*) apparently diagnosed as heterozygotes of *CYP2A6*1A* and *CYP2A6*4C*, Pharmacogenetics 10 (2000) 687–693.
[25] H. Kushida, K. Fujita, A. Suzuki, M. Yamada, T. Nohmi, T. Kamataki, Development of a *Salmonella* tester strain sensitive to promutagenic *N*-nitrosamines: expression of recombinant CYP2A6 and human NADPH-cytochrome P450 reductase in *S. typhimurium* YG7108, Mutat. Res. 471 (2000) 135–143.
[26] K. Fujita, T. Kamataki, Predicting the mutagenicity of tobacco-related *N*-nitrosamines in humans using 11 strains of *Salmonella typhimurium* YG7108, each coexpressing a form of human cytochrome P450 along with NADPH-cytochrome P450 reductase, Environ. Mol. Mutagen. 38 (2001) 339–346.
[27] H. Kushida, K. Fujita, A. Suzuki, M. Yamada, T. Endo, T. Nohmi, T. Kamataki, Metabolic activation of *N*-alkylnitrosamines in genetically engineered *Salmonella typhimurium* expressing CYP2E1 or CYP2A6 together with human NADPH-cytochrome P450 reductase, Carcinogenesis 21 (2000) 1227–1232.
[28] M. Miyamoto, Y. Umetsu, H. Dosaka-Akita, Y. Sawamura, J. Yokota, H. Kunitoh, N. Nemoto, K. Sato, N. Ariyoshi, T. Kamataki, *CYP2A6* gene deletion reduces susceptibility to lung cancer, Biochem. Biophys. Res. Commun. 261 (1999) 658–660.
[29] N. Ariyoshi, M. Miyamoto, Y. Umetsu, H. Kunitoh, H. Dosaka-Akita, Y. Sawamura, J. Yokota, N. Nemoto, K. Sato, T. Kamataki, Genetic polymorphism of *CYP2A6* gene and tobacco-induced lung cancer risk in male smokers, Cancer Epidemiol. Biomarkers Prev. 11 (2002) 890–894.
[30] N. Ariyoshi, Y. Sawamura, T. Kamataki, A novel single nucleotide polymorphism altering stability and activity of CYP2A6, Biochem. Biophys. Res. Commun. 281 (2001) 810–814.
[31] M. Pitarque, O. von Richter, B. Oke, H. Berkkan, M. Oscarson, M. Ingelman-Sundberg, Identification of a single nucleotide polymorphism in the TATA box of the *CYP2A6* gene: impairment of its promoter activity, Biochem. Biophys. Res. Commun. 284 (2001) 455–460.
[32] K. Kiyotani, H. Yamazaki, M. Fujieda, S. Iwano, K. Matsumura, S. Satarug, P. Ujjin, T. Shimada, F.P. Guengerich, A. Parkinson, G. Honda, K. Nakagawa, T. Ishizaki, T. Kamataki, Decreased coumarin 7-hydroxylase activities and CYP2A6 expression levels in humans caused by genetic polymorphism in *CYP2A6* promoter region (*CYP2A6*9*), Pharmacogenetics 13 (2003) 689–695.
[33] C. Xu, Y.S. Rao, B. Xu, E. Hoffmann, J. Jones, E.M. Sellers, R.F. Tyndale, An in vivo pilot study characterizing the new *CYP2A6*7*, *8, and *10 alleles, Biochem. Biophys. Res. Commun. 290 (2002) 318–324.
[34] E.L. Wynder, D. Hoffmann, Smoking and lung cancer: scientific challenges and opportunities, Cancer Res. 54 (1994) 5284–5295.
[35] L. Le Marchand, L. Sivaraman, L. Pierce, A. Seifried, A. Lum, L.R. Wilkens, A.F. Lau, Associations of CYP1A1, GSTM1, and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens, Cancer Res. 58 (1998) 4858–4863.

[36] M. Fujieda, H. Yamazaki, T. Saito, K. Kiyotani, M.A. Gyamfi, M. Sakurai, H. Dosaka-Akita, Y. Sawamura, J. Yokota, H. Kunitoh, T. Kamataki, Evaluation of *CYP2A6* genetic polymorphisms as determinants of smoking behavior and tobacco-related lung cancer risk in male Japanese smokers, Carcinogenesis 25 (2004) 2451–2458.

[37] H. Takeuchi, K. Saoo, M. Yokohira, M. Ikeda, H. Maeta, M. Miyazaki, H. Yamazaki, T. Kamataki, K. Imaida, 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 (2003) 7581–7583.