Single-nucleotide polymorphism (c.309T>G) in the *MDM2* gene and lung cancer risk

YASUAKI ENOKIDA[1], KIMIHIRO SHIMIZU[1], SEIICHI KAKEGAWA[1], JUN ATSUMI[1], YOSHIAKI TAKASE[1], YOHEI MIYAMAE[1], TOSHITERU NAGASHIMA[1], YOICHI OHTAKI[1], KYOICHI KAIRA[2], NORIAKI SUNAGA[3], NORIKO YANAGITANI[3], REIKO YOSHINO[4], KATSUHIKO TSUNEKAWA[5], HITOSHI IGAI[6], MITSUHIRO KAMIYOSHIHARA[6], KENGO USUI[7], ALEXANDER LEZHAVA[7], YOSHIO TOMIZAWA[4], TOSHIHISA ISHIKAWA[8], MASAMI MURAKAMI[5], YOSHIHIDE HAYASHIZAKI[9] and IZUMI TAKEYOSHI[1]

Departments of [1]Thoracic and Visceral Organ Surgery, [2]Oncology Clinical Development, and [3]Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511; [4]Department of Internal Medicine, National Hospital Organization Nishi-Gunma Hospital, Shibukawa, Gunma 377-8511; [5]Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511; [6]Department of General Thoracic Surgery, Maebashi Red Cross Hospital, Maebashi, Gunma 371-0014; [7]Division of Genomic Technologies, RIKEN Center for Life Science Technologies, Yokohama, Kanagawa 230-0045; [8]NGO Personalized Medicine and Healthcare, Yokohama, Kanagawa 226-0016; [9]RIKEN Preventive Medicine and Diagnosis Innovation Program, Yokohama, Kanagawa 230-0045, Japan

Received April 21, 2014; Accepted June 13, 2014

DOI: 10.3892/br.2014.305

Abstract. Murine double minute 2 (MDM2) is a negative regulator of p53. A single-nucleotide polymorphism (SNP) (rs2279744: c.309T>G) in the promoter region of the *MDM2* gene has been shown to result in higher levels of MDM2 RNA and protein. Regarding the contribution of c.309T>G in the *MDM2* gene to the lung cancer risk, previous studies are conflicting. In order to evaluate the association between c.309T>G and the lung cancer risk, a case-control study was performed. The *MDM2* genotypes were determined in 762 lung cancer patients and in 700 cancer-free control subjects using the Smart Amplification Process. Statistical adjustment was performed for gender, age and pack-years of smoking. The distributions of c.309T>G (T/T, T/G, G/G) were 20.1, 49.7, 30.2% in the case group and 21.7, 47.9, 30.4% in the healthy-control group. There were no overall associations between the *MDM2* genotypes and the risk of lung cancer [T/G genotype: Adjusted odds ratio (AOR), 1.30; 95% confidence interval (CI), 0.88-1.93; and G/G genotype: AOR, 1.18; 95% CI, 0.78-1.80]. The subgroup analysis of gender, histology, smoking status and epidermal growth factor receptor mutation status also indicated that there was no association with lung cancer. Additionally, the genotypes did not have an effect on the age at the time of diagnosis of lung cancer (P=0.25). In conclusion, the G allele frequency in the lung cancer cases was 0.551, which was similar to other studies. The results of the present study suggest that the c.309T>G is not significantly associated with lung cancer.

Correspondence to: Dr Kimihiro Shimizu, Department of Thoracic and Visceral Organ Surgery, Gunma University Graduate School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan
E-mail: kmshimizu@gmail.com

Abbreviations: SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval; PY, pack-year; AD, adenocarcinoma; SQ, squamous cell carcinoma; NSCLC, non-small cell lung cancer

Key words: lung cancer, *murine double minute 2*, *p53*, single-nucleotide polymorphism 309, smart amplification process, smoking history, cancer susceptibility

Introduction

Lung cancer is the leading cause of cancer death worldwide (1), and evidence indicates that cigarette smoking is the major established risk factor (2). Additionally, exposure to environmental-chemical carcinogens are other associated risk factors (3). The mechanism of lung tumorigenesis is not fully understood. Epidemiological evidence indicates that complex interactions between numerous genetic and environmental factors are significant in the carcinogenesis of lung carcinoma (4).

The *p53* gene is a well-known tumor suppressor gene that encodes a sequence-specific DNA-binding transcription factor that targets various genes that govern specific cellular processes (5). The murine double minute 2 (MDM2) protein plays an important role in regulating cell proliferation and apoptosis by transcriptional inhibition via direct physical binding to *p53* and ubiquitination and proteasome-mediated degradation of *p53* via its E3 ubiquitin ligase activity (6,7). A *MDM2* single nucleotide polymorphism at the 309th nucleotide in the first intron (rs2279744), with a T to G change, could increase the affinity for stimulatory protein 1

(Sp1) binding and result in increased MDM2 expression (8) and subsequent attenuation of the P53 pathway. In humans, the SNP (c.309T>G) in the *MDM2* gene has been correlated with the earlier onset of tumor formation in hereditary and sporadic cancers (9). A previous meta-analysis reported that the G/G genotype of c.309T>G was associated with a significantly increased risk for lung, colorectal, gastric and bladder cancers and a significantly decreased risk for prostate cancer (10). Molecular epidemiological studies of c.309T>G and lung cancer susceptibility (11-21) and survival rate (22-24) have reported disparate findings. It is noteworthy that there is a large difference among African-American, Caucasian and Asian populations with respect to the 309G allele frequency in lung cancer cases (25). The 309G allele is present at a higher frequency in the Asian population, including Japan, compared to Caucasians and African-Americans. Significant associations have been observed for c.309T>G in Japanese squamous cell lung cancer patients (20). Future studies analyzing the ethnic group-dependent susceptibility to cancer are required. Several genome-wide association studies (GWASs) on SNPs have identified the genomic loci associated with the risk of lung cancer in Caucasians (26-28), Europeans (29) and Asians (30-34). However, the associations of the *MDM2* polymorphisms were not investigated in the aforementioned GWASs due to a lack of probes used to discriminate between the polymorphisms (20).

The aim of the present study was to investigate whether a functionally important SNP, *MDM2* SNP309, was associated with the risk of lung cancer in a Japanese population. The genomic DNA was examined from blood samples for c.309T>G using the Smart Amplification Process (SmartAmp), which is a rapid, sensitive and simple mutation-detection assay that has been described previously (35,36). Subsequently, the association between c.309T>G and the lung cancer risk was examined. To the best of our knowledge, this is the first study to investigate the role of c.309T>G in the *MDM2* gene in all the types of lung cancer in a Japanese population.

Materials and methods

Subjects. The study included 762 patients with lung cancer and 700 cancer-free controls. All the cases were histologically confirmed primary lung cancer patients who were diagnosed and recruited between January 2003 and June 2013 at Gunma University Hospital, Maebashi Red Cross Hospital and National Nishi-Gunma Hospital (Gunma, Japan). The control group consisted of hospital and population controls. The hospital controls were recruited from the same hospital as the patients. The population controls were recruited from a health survey for the evaluation of metabolic syndrome in Gunma. The potential controls that had a previous diagnosis of malignancy were excluded from the study. The protocols for sample collection, sample anonymity, storage and genomic DNA analysis were approved by the Institutional Review Board for the Ethics Committee for Human Genome Analysis at each hospital. Written informed consent from all the participants was obtained. The clinical study was conducted according to the Declaration of Helsinki Principles.

Patient demographics. The demographics, cancer history and smoking history of the subjects were documented using a structured chart review. The patients were categorized based on the smoking status. The cumulative cigarette dose [pack-years (PY)] was calculated using the following formula: PY = packs per day x years smoked. Never-smokers were defined as individuals with PY=0, mild smokers were PY<50 and heavy smokers were PY≥50.

Table I. Characteristics of lung cancer cases and healthy controls.

Variable	Cases (n=762) n (%)	Controls (n=700) n (%)	P-value
Gender			<0.001[a]
Male	472 (61.9)	326 (46.6)	
Female	290 (38.1)	374 (53.4)	
Age, years			<0.001[b]
Mean ± SD	68.4±9.8	64.1±12.7	
Range	31-95	20-93	
MDM2 polymorphism			0.69[a]
T/T	153 (20.1)	152 (21.7)	
T/G	379 (49.7)	335 (47.9)	
G/G	230 (30.2)	213 (30.4)	
G allele frequency	0.551	0.544	
PY			
Mean ± SD	36.4±37.2	26.6±32.7	0.001[b]
Range	0-265	0-184	
Histology			
AD	487 (63.9)		
SQ	182 (23.9)		
Others	82 (10.8)		
Un-differentiated	11 (1.4)		

[a]Pearson's χ^2 test. [b]Kruskal-Wallis test. SD, standard deviation; *MDM2*, *murine double minute 2*; PY, pack-years; AD, adenocarcinoma; SQ, squamous cell carcinoma.

Genotyping for polymorphisms and gene mutation analysis. Peripheral venous blood samples were collected and DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genotyping of c.309T>G in the *MDM2* gene was performed by the Duplex SmartAmp method as described previously (36) with an Mx3000P PCR system (Agilent Technologies, Santa Clara, CA, USA). The lung cancer tissues were immediately frozen following surgical removal and stored at -80˚C until DNA extraction using a Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA). The epidermal growth factor receptor (*EGFR*) mutations were analyzed by peptide nucleic acid-enriched sequencing, as described previously (37).

Statistical analyses. The demographic and clinical information were compared across genotypes and cancer stages using Pearson's χ^2 tests (for categorical variables) and Kruskal-Wallis tests (for continuous variables) where appropriate. The

Table II. Genotype frequencies of the *MDM2* 309T>G polymorphism among controls and cases and their association with lung cancer risk.

					Adjusted OR (95% CI)[a]		
Variable	No.	T/T (%)	T/G (%)	G/G (%)	T/T vs. G/G	T/T vs. T/G+G/G	T/T+T/G vs. G/G
Control	700	152 (21.7)	335 (47.9)	213 (30.4)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Case	762	153 (20.1)	379 (49.7)	230 (30.2)	1.18 (0.76-1.80)	1.25 (0.87-1.81)	0.98 (0.71-1.37)
Female[b]	290	53 (18.3)	143 (49.3)	94 (32.4)	1.31 (0.91-1.87)	1.51 (0.83-2.75)	1.34 (0.76-2.34)
Male[b]	472	100 (21.2)	236 (50.0)	136 (28.8)	0.94 (0.55-1.60)	1.13 (0.72-1.79)	0.83 (0.55-1.24)
AD	487	101 (20.7)	233 (47.8)	153 (31.4)	1.05 (0.84-1.31)	1.14 (0.77-1.67)	1.02 (0.72-1.44)
SQ	182	35 (19.2)	98 (53.8)	49 (26.9)	1.06 (0.77-1.46)	1.42 (0.80-2.49)	0.87 (0.52-1.43)
EGFR-WT-AD	207	41 (19.8)	102 (49.3)	64 (30.9)	1.09 (0.83-1.42)	1.28 (0.80-2.05)	1.01 (0.67-1.52)
EGFR-Mt-AD	175	39 (22.3)	78 (44.6)	58 (33.1)	1.00 (0.75-1.35)	0.97 (0.58-1.61)	1.04 (0.66-1.64)
Never-smoker	251	43 (17.1)	129 (51.4)	79 (31.5)	1.07 (0.75-1.55)	1.46 (0.78-2.73)	0.89 (0.52-1.54)
Light smoker	258	49 (19.0)	127 (49.2)	82 (31.8)	1.09 (0.85-1.41)	1.30 (0.83-2.03)	1.00 (0.68-1.49)
Heavy smoker	246	57 (23.2)	120 (48.8)	69 (28.0)	1.00 (0.71-1.41)	1.07 (0.60-1.92)	0.95 (0.55-1.64)

[a]Adjusted for gender, age and smoking status. [b]Adjusted for age and smoking status. *MDM2*, *murine double minute 2*; OR, odds ratio; CI, confidence interval; AD, adenocarcinoma; SQ, squamous cell carcinoma; *EGFR*, *epidermal growth factor receptor*; WT, wild-type; Mt; mutant.

strengths of the associations between the genotypes and risk were measured as odds ratios (ORs) adjusted for gender, age and PY of smoking with 95% confidence intervals (CIs) by unconditional logistic regression analysis. All the statistical analyses were conducted at the two-sided P=0.05 level and SPSS Statistics version 20 (SPSS, Inc., Chicago, IL, USA) was used. P<0.05 was considered to indicate a statistically significant difference.

Results

Demographics. A total of 762 primary lung cancer patients and 700 controls were included in the analysis and Table I shows the associated demographics. Compared to the controls, the patients were older with a higher proportion of males. The mean PY value was higher in the cases compared to the controls (Table I). Complete matching was not achieved for gender, age and smoking history. Therefore, these factors were adjusted in the logistic model. The frequency of c.309T>G did not differ between the hospital and population controls (P=0.74; data not shown). Therefore, the controls were treated as a single group. The distributions of c.309T>G (T/T, T/G, G/G) were 20.1, 49.7, 30.2% in the patient group and 21.7, 47.9, 30.4% in the healthy-control group. There were no significant differences in the c.309T>G genotypes between cases and controls. The allele frequencies of the G allele in the case and control groups were 0.551 and 0.544, respectively.

MDM2 and lung cancer risk. There was no overall association between c.309T>G and the risk of lung cancer (Table II). The adjusted OR for developing lung cancer was 1.18 (95% CI, 0.76-1.80) for the G/G, compared to the T/T. The data was further stratified by gender, cancer histology, *EGFR*-mutation status of adenocarcinoma (AD) and smoking-status subgroups. There were no associations among any subgroup (Table II).

MDM2 and cigarette smoking. As smoking is a stressor and an established cause of lung cancer, we evaluated whether an interaction existed between the *MDM2* polymorphism and smoking among lung cancer patients. No significant associations were observed between the various c.309T>G genotypes and cumulative smoking. (Table III). However, the G/G genotype tended to be associated with a lower level of exposure to cigarette smoke compared to the other genotype (P=0.07) among squamous cell carcinoma (SQ) patients (Table III).

MDM2 and age at diagnosis. The association between age at diagnosis and c.309T>G in all the lung cancer patients was examined, as well as in the gender, histological-subtype and smoking-status subgroups. Kruskal-Wallis analysis indicated no association between the various c.309T>G genotypes and age at onset in the study population (Table IV).

Discussion

In the present molecular epidemiological study, the association of the genetic polymorphisms in *MDM2* with the risk of developing lung cancer in a Japanese population was examined. The results obtained by analyzing 762 lung cancer patients and 700 controls demonstrated that c.309T>G in the *MDM2* gene is not associated with lung cancer risk.

In the study, the frequency of the *MDM2* 309G alleles among the controls was 0.544, which is almost identical to the frequency observed in the Japanese (0.525) (20), Han Chinese of southeast China (0.499) (14) and Korean (0.534) (16) populations; but is significantly higher compared to the Han Chinese of northeast China (0.455) (15), Caucasian (0.346-0.393) (11-13,18,38) and African-American (0.109-0.112) (13,38) populations (Fig. 1). There are conflicting studies regarding the contribution of c.309T>G to the lung cancer risk (11-19,21). The differences in the allele frequencies among ethnic groups may have contributed to the disparities among the previous studies.

Table III. Comparison by cumulative smoking.

Variable	n	T/T Mean ± SD	T/G Mean ± SD	G/G Mean ± SD	P-value[a] (T/T+T/G vs. G/G)
Total	755	39.9±36.5	36.5±38.7	34.1±35.0	0.35
Female	286	8.2±18.7	8.2±18.6	7.6±18.4	0.88
Male	469	55.9±32.6	53.6±37.6	52.5±31.9	0.70
AD	482	26.3±30.1	20.6±28.8	23.1±31.4	0.99
SQ	182	70.8±33.9	62.0±32.9	57.2±34.5	0.07
SCLC	52	58.8±34.9	67.5±56.7	59.2±24.8	0.93

[a]Kruskal-Wallis test. SD, standard deviation; AD, adenocarcinoma; SQ, squamous cell carcinoma; SCLC, small cell lung cancer.

Table IV. Comparison by age at diagnosis.

Variable	n	T/T Mean ± SD	T/G Mean ± SD	G/G Mean ± SD	P-value[a] (T/T+T/G vs. G/G)
Total	761	67.4±9.6	68.9±9.7	68.2±10.0	0.54
Female	289	66.3±9.7	67.2±10.2	66.7±10.4	0.82
Male	472	68.0±9.6	69.9±9.3	69.2±9.5	0.69
AD	487	66.7±10.3	67.2±10.3	66.9±10.2	0.67
SQ	182	71.0±6.3	72.8±7.9	72.0±9.1	0.86
SCLC	52	64.9±9.9	69.3±7.8	70.8±8.9	0.27
Smoker	507	68.3±9.5	69.7±9.7	69.0±10.0	0.60
Never smoker	251	65.6±9.8	67.4±9.6	66.7±9.9	0.82

[a]Kruskal-Wallis test. SD, standard deviation; AD, adenocarcinoma; SQ, squamous cell carcinoma; SCLC, small cell lung cancer.

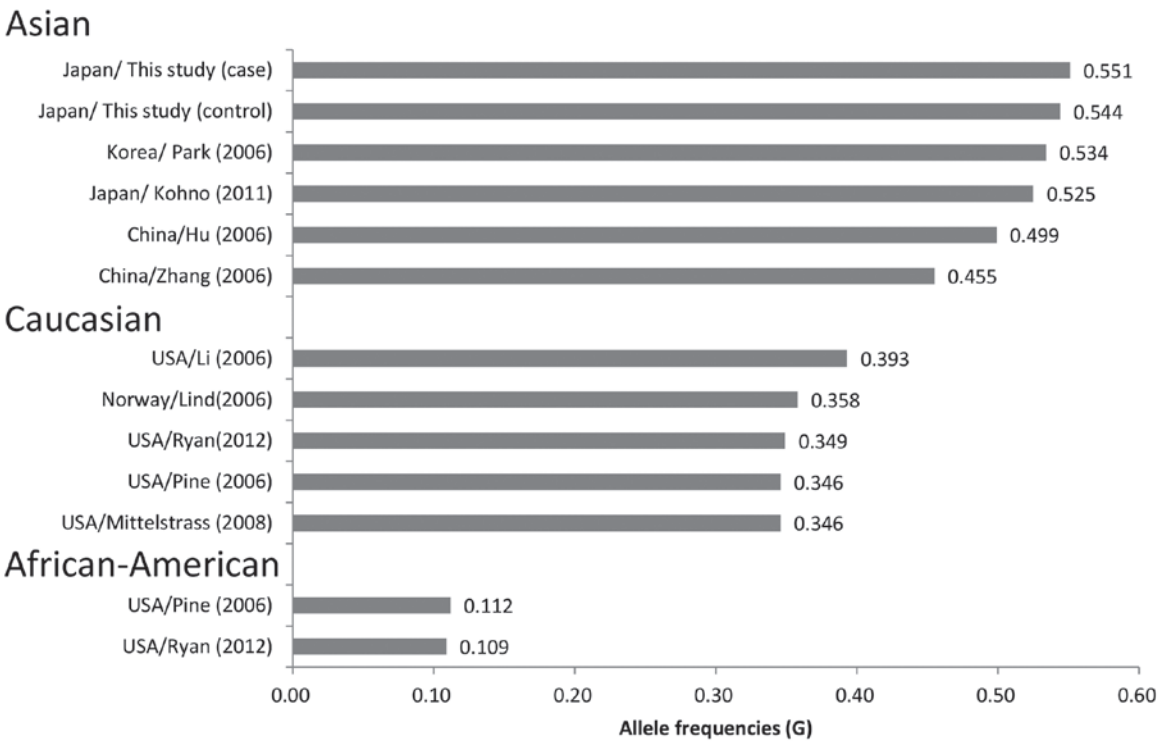

Figure 1. Comparison of the allele frequencies of the 309 G allele.

Certain studies have linked the G allele with the lung cancer risk among Caucasians (11) and Asians (15). Conversely, only one study linked the 309T allele with the risk in Caucasians, notably in SQ patients and in males (12). The data of the present study suggests that c.309T>G is not associated with the lung cancer risk among the total population, which is in line with numerous epidemiological studies conducted in Caucasians (13,17,18), African-Americans (13) and Asians (14,16,20). A previous meta-analysis study (39) concluded that the association between c.309T>G and lung cancer was statistically significant in Asians, females and never-smokers, but not in Caucasians, males, ever-smokers or among any of the individual histological types.

Stratification by gender, histological subtype and smoking status revealed no association between c.309T>G and lung cancer susceptibility. Lind *et al* (11) reported a gender-specific risk-disposing effect of the 309T allele of *MDM2* SNP309 for non-small cell lung cancer (NSCLC) patients. NSCLC patients were investigated and it was found that female carriers of G/G had an OR for lung cancer of 4.1, whereas male homozygotes had a non-significant OR of 1.3. The data of the present study showed corresponding ORs for females and males of 1.31 and 0.94, respectively, which tended to confirm this finding, although the ORs were much smaller and not significant. Bond *et al* (40) showed that the effect of the *MDM2* G/G genotype was gender-specific and was increased in females with active estrogen-signaling pathways, which may explain the findings of the present study.

In terms of histological subtypes, Park *et al* (16) and Ren *et al* (21) reported that the T/T genotype was associated with a decreased risk of AD. However, the present study findings, as well as those of others (18,41), did not show an interaction between c.309T>G and the histological subtype.

Cigarette smoking may induce DNA damage, initiating and promoting carcinogenesis, and is a major risk factor for NSCLC. Park *et al* (16) and Zhang *et al* (15) reported that the T/T genotype was associated with a decreased risk among ever-smokers and ever- and never-smokers, respectively. However, the present study, as well as others (18,41), did not show an interaction between c.309T>G in the *MDM2* gene and smoking status. A tendency towards a lower level of exposure to cigarette smoking was observed among the G/G SQ patients compared with T allele carriers (T/T+T/G), but the difference was not statistically significant. However, no significant association between c.309T>G and the lung cancer risk among smokers or SQ patients was observed. Therefore, it was concluded that SNP309 did not contribute to smoking-related tumors.

c.309T>G was reported to be associated with an earlier onset of disease for Li-Fraumeni syndrome and sporadic sarcoma (8). The present study did not observe a lower age of diagnosis, and similar findings were described in previous studies (13,14,18).

Although the reason for the differences in *MDM2* polymorphisms among different studies is unclear, it may be due to differences in subjects, genetic backgrounds and/or environmental factors (42) among various populations. As an example of population heterogeneity, an interaction of c.309T>G with c.285G>C in the *MDM2* gene was questionable (43), as it has been previously reported to act as an antagonist by overriding the effect of c.309T>G on Sp1-mediated transcription. However, the study by Ryan *et al* (38) recently reported that c.285G>C did not antagonize the effect of c.309T>G in lung cancer. Furthermore, the differences in frequencies of driver mutations, such as *EGFR* mutations (44), may contribute to the different effects of c.309T>G in various ethnic groups.

EGFR mutations are predominantly found in female, non-smoking AD patients and in patients of East Asian origin (45). A recent meta-analysis study (39) reported that the association between c.309T>G and lung cancer was statistically significant, particularly in the Asian population, female and never-smokers. No statistically significant association was observed in males or ever-smokers. The AD patients were stratified according to *EGFR*-mutation status, however, no association was observed. To the best of our knowledge, there have been no report in terms of analyzing the association between c.309T>G in the *MDM2* gene and lung cancer susceptibility based on *EGFR* mutation status.

The limitations of the study include the retrospective nature, and the patient populations may be biased. Furthermore, the study was a hospital- and community-based case-control study, which could not rule out possible selection bias. However, by matching age, gender and PY of smoking, the potential confounding effects should have been reduced. To further elucidate the impact of c.309T>G on lung cancer susceptibility, future investigations of large ethnically-diverse population-based studies are warranted.

In conclusion, the present study is the first to evaluate the overall lung cancer risk and c.309T>G in a Japanese population. Considering the contradictory results across multiple studies, conclusions from any single study must be interpreted with caution. The results of the present study should be confirmed by other prospective studies.

Acknowledgements

The present study was supported by Grant-in-Aid for Scientific Research (C) (grant no. 22590516) from the Japan Society for the Promotion of Science. The study was partially supported by i) research grants for the RIKEN Omics Science Center from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) to Y.H.; and ii) a research grant from the MEXT to the RIKEN Center for Life Science Technologies. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
2. Steliga MA and Dresler CM: Epidemiology of lung cancer: smoking, secondhand smoke, and genetics. Surg Oncol Clin N Am 20: 605-618, 2011.
3. Fucic A, Gamulin M, Ferencic Z, *et al*: Lung cancer and environmental chemical exposure: a review of our current state of knowledge with reference to the role of hormones and hormone receptors as an increased risk factor for developing lung cancer in man. Toxicol Pathol 38: 849-855, 2010.
4. Yokota J, Shiraishi K and Kohno T: Genetic basis for susceptibility to lung cancer: Recent progress and future directions. Adv Cancer Res 109: 51-72, 2010.
5. Jin S and Levine AJ: The p53 functional circuit. J Cell Sci 114: 4139-4140, 2001.

6. Momand J, Zambetti GP, Olson DC, George D and Levine AJ: The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 69: 1237-1245, 1992.
7. Thut CJ, Goodrich JA and Tjian R: Repression of p53-mediated transcription by MDM2: a dual mechanism. Genes Dev 11: 1974-1986, 1997.
8. Bond GL, Hu W, Bond EE, *et al*: A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 119: 591-602, 2004.
9. Bond GL, Hu W and Levine A: A single nucleotide polymorphism in the MDM2 gene: from a molecular and cellular explanation to clinical effect. Cancer Res 65: 5481-5484, 2005.
10. Wo X, Han D, Sun H, *et al*: MDM2 SNP309 contributes to tumor susceptibility: a meta-analysis. J Genet Genomics 38: 341-350, 2011.
11. Lind H, Zienolddiny S, Ekstrøm PO, Skaug V and Haugen A: Association of a functional polymorphism in the promoter of the MDM2 gene with risk of nonsmall cell lung cancer. Int J Cancer 119: 718-721, 2006.
12. Li G, Zhai X, Zhang Z, Chamberlain RM, Spitz MR and Wei Q: MDM2 gene promoter polymorphisms and risk of lung cancer: a case-control analysis. Carcinogenesis 27: 2028-2033, 2006.
13. Pine SR, Mechanic LE, Bowman ED, *et al*: MDM2 SNP309 and SNP354 are not associated with lung cancer risk. Cancer Epidemiol Biomarkers Prev 15: 1559-1561, 2006.
14. Hu Z, Ma H, Lu D, *et al*: Genetic variants in the MDM2 promoter and lung cancer risk in a Chinese population. Int J Cancer 118: 1275-1278, 2006.
15. Zhang X, Miao X, Guo Y, *et al*: Genetic polymorphisms in cell cycle regulatory genes MDM2 and TP53 are associated with susceptibility to lung cancer. Hum Mutat 27: 110-117, 2006.
16. Park SH, Choi JE, Kim EJ, *et al*: MDM2 309T>G polymorphism and risk of lung cancer in a Korean population. Lung Cancer 54: 19-24, 2006.
17. Liu G, Wheatley-Price P, Zhou W, *et al*: Genetic polymorphisms of MDM2, cumulative cigarette smoking and nonsmall cell lung cancer risk. Int J Cancer 122: 915-918, 2008.
18. Mittelstrass K, Sauter W, Rosenberger A, *et al*: Early onset lung cancer, cigarette smoking and the SNP309 of the murine double minute-2 (MDM2) gene. BMC Cancer 8: 113, 2008.
19. Chua HW, Ng D, Choo S, *et al*: Effect of MDM2 SNP309 and p53 codon 72 polymorphisms on lung cancer risk and survival among non-smoking Chinese women in Singapore. BMC Cancer 10: 88, 2010.
20. Kohno T, Kunitoh H, Mimaki S, et al: Contribution of the TP53, OGG1, CHRNA3, and HLA-DQA1 genes to the risk for lung squamous cell carcinoma. J Thorac Oncol 6: 813-817, 2011.
21. Ren YW, Yin ZH, Wan Y, *et al*: P53 Arg72Pro and MDM2 SNP309 polymorphisms cooperate to increase lung adenocarcinoma risk in Chinese female non-smokers: a case control study. Asian Pac J Cancer Prev 14: 5415-5420, 2013.
22. Heist RS, Zhou W, Chirieac LR, *et al*: MDM2 polymorphism, survival, and histology in early-stage non-small-cell lung cancer. J Clin Oncol 25: 2243-2247, 2007.
23. Han JY, Lee GK, Jang DH, Lee SY and Lee JS: Association of p53 codon 72 polymorphism and MDM2 SNP309 with clinical outcome of advanced nonsmall cell lung cancer. Cancer 113: 799-807, 2008.
24. Dong J, Ren B, Hu Z, *et al*: MDM2 SNP309 contributes to non-small cell lung cancer survival in Chinese. Mol Carcinog 50: 433-438, 2011.
25. Zhuo W, Zhang L, Zhu B, Ling J and Chen Z: Association of MDM2 SNP309 variation with lung cancer risk: evidence from 7196 cases and 8456 controls. PLoS One 7: e41546, 2012.
26. Landi MT, Chatterjee N, Yu K, *et al*: A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. Am J Hum Genet 85: 679-691, 2009.
27. Hung RJ, McKay JD, Gaborieau V, *et al*: A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature 452: 633-637, 2008.
28. Wang Y, Broderick P, Webb E, *et al*: Common 5p15.33 and 6p21.33 variants influence lung cancer risk. Nat Genet 40: 1407-1409, 2008.
29. Shi J, Chatterjee N, Rotunno M, *et al*: Inherited variation at chromosome 12p13.33, including RAD52, influences the risk of squamous cell lung carcinoma. Cancer Discov 2: 131-139, 2012.
30. Hosgood HD III, Wang WC, Hong YC, *et al*: Genetic variant in TP63 on locus 3q28 is associated with risk of lung adenocarcinoma among never-smoking females in Asia. Hum Genet 131: 1197-1203, 2012.
31. Ahn MJ, Won HH, Lee J, *et al*: The 18p11.22 locus is associated with never smoker non-small cell lung cancer susceptibility in Korean populations. Hum Genet 131: 365-372, 2012.
32. Hu Z, Wu C, Shi Y, *et al*: A genome-wide association study identifies two new lung cancer susceptibility loci at 13q12.12 and 22q12.2 in Han Chinese. Nat Genet 43: 792-796, 2011.
33. Shiraishi K, Kunitoh H, Daigo Y, *et al*: A genome-wide association study identifies two new susceptibility loci for lung adenocarcinoma in the Japanese population. Nat Genet 44: 900-903, 2012.
34. Lan Q, Hsiung CA, Matsuo K, *et al*: Genome-wide association analysis identifies new lung cancer susceptibility loci in never-smoking women in Asia. Nat Genet 44: 1330-1335, 2012.
35. Mitani Y, Lezhava A, Kawai Y, *et al*: Rapid SNP diagnostics using asymmetric isothermal amplification and a new mismatch-suppression technology. Nat Methods 4: 257-262, 2007.
36. Enokida Y, Shimizu K, Atsumi J, *et al*: Rapid detection of SNP (c.309T>G) in the MDM2 gene by the Duplex SmartAmp method. PLoS One 8: e60151, 2013.
37. Miyamae Y, Shimizu K, Mitani Y, *et al*: Mutation detection of epidermal growth factor receptor and KRAS genes using the smart amplification process version 2 from formalin-fixed, paraffin-embedded lung cancer tissue. J Mol Diagn 12: 257-264, 2010.
38. Ryan BM, Calhoun KM, Pine SR, *et al*: MDM2 SNP285 does not antagonize the effect of SNP309 in lung cancer. Int J Cancer 131: 2710-2716, 2012.
39. He W, Long J, Xian L, *et al*: MDM2 SNP309 polymorphism is associated with lung cancer risk in women: A meta-analysis using METAGEN. Exp Ther Med 4: 569-576, 2012.
40. Bond GL, Hirshfield KM, Kirchhoff T, *et al*: MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. Cancer Res 66: 5104-5110, 2006.
41. Liu JN, Zhang XM, Guo YL, Sun T, Lin DX and Wen T: Genetic polymorphism in MDM2 is associated with susceptibility to colorectal cancer in a Chinese population. Zhonghua Zhong Liu Za Zhi 30: 335-338, 2008 (In Chinese).
42. Alberg AJ, Ford JG and Samet JM; American College of Chest Physicians: Epidemiology of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). Chest 132 (Suppl 3): 29S-55S, 2007.
43. Knappskog S, Bjørnslett M, Myklebust LM, *et al*: The MDM2 promoter SNP285C/309G haplotype diminishes Sp1 transcription factor binding and reduces risk for breast and ovarian cancer in Caucasians. Cancer Cell 19: 273-282, 2011.
44. Nana-Sinkam SP and Powell CA: Molecular biology of lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest 143 (5 Suppl): e30S-e39S, 2013.
45. Mitsudomi T and Yatabe Y: Mutations of the epidermal growth factor receptor gene and related genes as determinants of epidermal growth factor receptor tyrosine kinase inhibitors sensitivity in lung cancer. Cancer Sci 98: 1817-1824, 2007.