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Impact of MDM2 SNP309T>G Polymorphism: Increased Risk of developing Non Small Cell Lung Cancer and Poor Prognosis in Indian Patients

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Abstract

Background: MDM2 is an important negative regulator of the TP53 pathway, over expressed in many cancers as oncoprotein. Polymorphisms in the promoter region of the MDM2 gene have been shown to alter protein expression and may, thus play an important role in carcinogenesis.

Aim and methods: To test our hypothesis that the MDM2 promoter polymorphisms are associated with risk of non small cell lung cancer, we conducted a hospital-based, case–control study of 136 Indian patients diagnosed with NSCLC and 136 cancer-free controls and investigated the association between genetic variation in the promoter region of MDM2 (c.–51309G4T, rs2279744:g.G4T) and the risk of developing NSCLC by tetra-primer ARMS-PCR and ASO-PCR.

Results: Compared with the MDM2-2580TT genotype, we found that the MDM2-309G variant genotypes were associated with an increased risk of NSCLC in Indian patients [OR 3.88 (1.82-8.27) RR 1.94 (1.27-2.96) RD 32.6 (15.7-49.6) p 0.0004 for GG and OR 2.60 (1.49-4.57) RR 1.52 (1.20-1.93) RD 23.16 (10.3-36.0) p 0.0009 for GT genotype]. GG genotype was found to be associated with poor survival outcome of NSCLC patients and in addition significant association was observed with stage (p 0.01) and metastasis status (p 0.002) of NSCLC patients.

Conclusion: Genetic polymorphism in cell cycle regulatory genes MDM2 contribute to the risk of developing NSCLC in Indian Patients. In addition G allele was associated with an increased risk and poor survival outcome than T allele.

Keywords: MDM2; SNP309T>G polymorphism; Non small cell lung cancer; Tetra-primer ARMS-PCR and ASO-PCR; Amplification refractory mutation system; Allele specific oligonucleotide

Abbreviations: MDM2: Murine Double Minute 2; ARMS: Amplification Refractory Mutation System; ASO: Allele Specific Oligonucleotide; NSCLC: Non Small Cell Lung Cancer

Introduction

Lung cancer, the most common form of malignancy leading to the major cause of cancer related deaths around the world [1] including India. Lung cancer constituted 14.4% of all cancers in a review of 9210 consecutive autopsies by Banker [2]. Cigarette smoking constitutes 80% of the attributable risk to lung cancer, but only a small proportion of smokers will develop lung cancer, suggesting that there is an interindividual variation in genetic susceptibility to lung cancer in the general population [1]. The human homologue of MDM2 located on chromosome 12q13-14 with genomic size of 34 kb is a negative regulator of the tumor suppressor gene p53 [3]. The encoded protein is a nuclear phosphoprotein that binds to p53 and inhibits p53-dependent transcription [4]. Over expression of this gene can result in excessive inactivation of p53, which enables damaged cells to escape the cell-cycle checkpoint control and become carcinogenic [5,6]. MDM2 has also been shown to promote tumor growth in a p53-independent manner through interaction with transcriptional factors of the E2F family [7], inhibition of the Rb growth regulatory function [8] and inhibition of G0/G1-S-phase transition in normal cells [9]. In tumors, the overexpression of MDM2 mRNA and proteins can substitute for p53 inactivation in the absence of p53 mutations [10] and is thus, often associated with the clinical behavior of the tumors, such as cancer progression and treatment response [11].

MDM2 polymorphisms in a regulatory region, such as the

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promoter, may alter its transcriptional activities, there by affecting p53 tumor suppression and carcinogenesis in humans. Therefore, MDM2 functional promoter polymorphisms probably contribute to inter individual variation in susceptibility to lung cancers as evidenced by an early age of onset [12]. Thus present study aimed to investigate the effect of the MDM2 promoter polymorphism (309T/G) on the risk and susceptibility of NSCLC in Indian patients.

Materials and Methods

Study population

Clinically confirmed NSCLC patients were selected from an on going molecular study of NSCLC Conducted in Department of Biochemistry, Maulana Azad Medical College & associated hospitals, New Delhi. The study includes 136 NSCLC patients and 136 healthy controls frequency matched with age (±5years) and gender. Patients with a history of previous cancer or metastasized cancer from other organs except lung were excluded. All controls, like the cases, were the residents of North India. The study was approved by the institutional ethics committee, MAMC, New Delhi. Written informed consent was obtained from all subjects. Patient follow up was obtained through of hospital records as well as by direct patient contact.

Genotype analysis

Genomic DNA was extracted from blood samples using DNA sure blood mini kit (Nucleo-pore Genetix brand) according to the manufacturer's protocol. MDM2 (NM_ 002392.2) c.-5+309T/G, rs 2279744:g.T/G genotypes were analyzed using ASO [13] and tetra primer ARMS-PCR [14]. Genotyping was performed without the knowledge of the case/control status of the study subjects. The amplification was accomplished with a 25 ul reaction mixture containing 5 ul of 20 ng template DNA, 0.25 ul of 25 pmol each Primers, 2.5 ul 10 mM dNTPs, 1.5 ul of 20 mM $MgCl_2$, 0.3 ul of 5 U/ul Taq polymerase with 2.5 ul of 10X Taq Buffer (Fermantas). The amplification conditions were 10 min of initial denaturation at 95°C; 40 cycles at 95°C for 45s, 66.7°C for 45s and 72°C for 45s with a final 10 min extension step at 72°C. ASO- PCR amplification reaction and conditions were same as above except 60°C of annealing temperature with ASO specific. PCR products were visualized on ethidium bromide stained 2% agarose gel (Figures 1A and 1B).

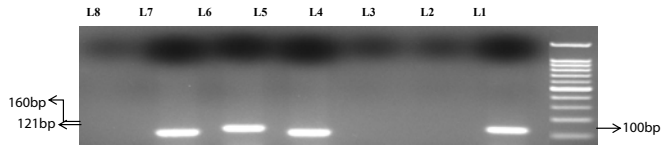

Figure 1a: ASO PCR MDM2 T309G: L–100bp ladder, L2, L–Homozygous TT, L4, L5-Homozygous GG, L6, L7–Heterozygous GT, and L8–Negative control.

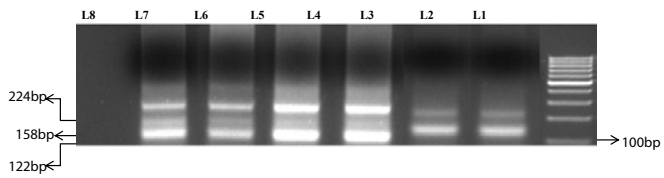

Figure 1b: ARMS PCR MDM2 T309G: L1-100bp ladder, L2, L3-Homozygous GG, L4, L5–Homozygous TT, L6, L7-Heterozygous GT and L8–Negative control.

Variable	NSCLC patients (%)	Healthy Controls (%)
Total No.	136	136
Sex		
Males	112(82.3)	112(82.3)
Females	24(17.7)	24(17.7)
Age (Years)		
≤45	22(16.2)	22(16.2)
>45	114(83.8)	114(83.8)
Mean ± SD age (Y)	57.39 ±10.9	56 ±10.3
Smoking Status		
Non Smoker	35(24.3)	136(100)
Smokers	101(75.7)	
Current smokers	40(39.6)	
Ex-smokers	61(60.4)	
Smoking Level (Pack Year)		
Mild (≤10)	10(9.9)	
Moderate (≤40)	45(44.6)	
Heavy (>40)	46(45.5)	
Histological Type		
SCC	79(58.1)	
ADC	57(41.9)	
TNM Stage		
Early (I & II)	51(37.5)	
Advanced (III & IV)	85(62.5)	
Distant Metastasis		
Positive	34(25.0)	
Negative	102(75.0)	
Family History of any Cancer		
Significant	18(13.2)	
Non Significant	118(86.8)	
Smoking Type		
Cigarette	29(28.7)	
Bidi	13(12.9)	
Huka	26(25.7)	
Cigarette+Bidi	11(10.9)	
Cigarette+Huka	22(21.8)	
Cytological Type		
Squamous cell carcinoma		
Well differentiated	43(54.4)	
Moderately Differentiated	22(27.8)	
Poorly Differentiated	14(17.7)	
Adenocarcinoma		
Well differentiated	11(19.3)	
Moderately Differentiated	16(28.1)	
Poorly Differentiated	30(52.6)	

Table 1: Distribution of Selected Characteristics among NSCLC patients.

Statistical analysis

Differences in select demographic variables and MDM2 genotype frequencies between the cases and controls were evaluated by using the Chi–square test. The associations between MDM2 variant genotypes and risk of lung cancer were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from both univariate and multivariate logistic regression analyses. Fisher Exact Test was performed for the values below 5. Statistical difference was considered significant for P values<0.05.

Results

General characteristics of study population

The demographic variables among cases and healthy controls are summarized in (Table 1). Briefly among NSCLC cases 112 were male and only 24 were females. Smokers constitute more than 75 percent with 39.6% as current and 60.4 as ex-smokers, who had left smoking from more than 6 months. Only few patients (13.2%) were with some significant family history of lung cancer or any other cancer. Histological studies revealed 79 cases with Squamous cell carcinoma, 57 cases with adenocarcinoma. Two age groups were made, patients with age <45 included 24 cases and >45 included 112 cases. More than 60% of the patients were in advanced stages with 25% cases positive for distant metastasis.

Allele and genotype distribution

The genotyping results are shown in Table 2A. The frequencies of MDM2-TT, -TG, and -GG genotypes among patients were

Genotype	TT	TG	GG	T allele frequency	G allele frequency
Patients (n=136)	28(20.6)	78(57.4)	30(22.0)	0.49	0.51
Controls (n=136)	58(42.6)	62(45.6)	16(11.8)	0.65	0.35
Chi-Square=16.55	df=2	P<0.0003			

Table 2A: Genotype frequencies of MDM2 309 T/G among NSCLC cases and controls.

Variables	TT Genotype n(%)	TG Genotype n(%)	GG Genotype n(%)	T allele frequency	G allele frequency	Chi-Square	df	P –value
Gender								
Males	26(23.2)	65(58.0)	21(18.8)	0.52	0.48	5.3	2	0.06
Females	2(8.3)	13(54.2)	9(37.5)	0.35	0.65			
Age at diagnosis (Years)								
≤45	3(13.6)	15(68.2)	4(18.2)	0.48	0.52			0.56
>45	25(21.9)	63(55.3)	26(22.8)	0.5	0.5			
Histological Type								
SCC	19(24.1)	41(51.8)	19(24.1)	0.5	0.5	2.41	2	0.29
ADC	9(15.8)	37(64.9)	11(19.2)	0.48	0.52			
TNM Stage								
Early Stage (I&II)	10(19.6)	36(70.6)	5(9.8)	0.55	0.45	8.09	2	0.01
Advanced Stage (III& IV)	18(21.1)	42(49.5)	25(29.4)	0.46	0.54			
Metastasis								
Positive	10(29.4)	11(32.4)	13(38.2)	0.46	0.54	12.03	2	0.002
Negative	18(17.6)	67(65.7)	17(16.7)	0.5	0.5			
Family History of any Cancer								
	9(50.0)	8(44.4)	1(5.6)	0.72	0.28			0.004
	19(16.1)	70(59.3)	29(24.6)	0.46	0.54			
Smoking Status								
Non Smoker	4(11.4)	24(68.6)	7(20.0)	0.46	0.54	3.05	2	0.22
Smokers	24(23.7)	54(53.5)	23(22.8)	0.5	0.5			
Smoking Level (Pack Year)								
Mild (≤10)	0(0)	9(90.0)	1(10.0)	045	0.55	10.3	4	0.036
Moderate (≤40)	10(22.2)	27(60.0)	8(17.8)	0.52	0.48			
Heavy (>40)	14(30.4)	18(39.0)	14(30.4)	0.5	0.5			
Smoking Type								
Cigarette	8(27.6)	16(55.2)	5(17.2)	0.55	0.45			0.99
Huka	7(27.0)	14(53.8)	5(19.2)	0.54	0.46			
Bidi	3(23.0)	8(61.6)	2(15.4)	0.54	0.46			
Cigarette+Bidi	1(9.0)	4(36.4)	6(54.6)	0.27	0.73			0.08
Cigarette+Huka	9(40.9)	12(54.5)	5(22.7)	0.58	0.42			
Cytological Type								
Squamous Cell Carcinoma								
Well Differentiated	11(25.5)	22(51.2)	10(23.3)	0.51	0.49			0.56
Moderately Differentiated	7(31.8)	10(45.5)	5(22.7)	0.55	0.45			
Poorly Differentiated	1(7.1)	9(64.3)	4(28.6)	0.39	0.61			
Adenocarcinoma								
Well Differentiated	2(18.2)	9(81.8)	0(0)	0.59	0.41			0.07
Moderately Differentiated	0(0)	12(75.0)	4(25.0)	0.38	0.63			
Poorly Differentiated	7(23.3)	16(53.3)	7(23.3)	0.5	0.5			

Table 2B: Associations and stratification analysis of MDM2 309T/G polymorphism and NSCLC.

significantly different compared to healthy controls (Chi square-16.6, df 2 & p-0.0003), with the GG homozygotes being significantly overrepresented among patients compared to controls (22.0% vs.11.8%) The frequency of the genotypes TT, TG and GG among healthy controls were 42.6%, 45.6% and 11.8% and NSCLC patients were 20.6%, 57.4% and 22.0% respectively. The frequencies of MDM2-TT, -TG, and -GG genotypes with respect to TNM stage (p 0.01) and metastasis status (p 0.002) among patients were statistically significant. whereas their was not any significant difference with respect to other parameter's like gender, age and Smoking status (Table 2B).

MDM2 309 T/G polymorphism and NSCLC risk

An unconditional logistic regression was used to estimate associations between the genotypes and risk of lung cancer (Table 3). It was found that an increased risk of lung cancer was associated with the MDM2 G allele in an allele dosage-dependent manner. Compared to the TT genotype, the ORs 2.60 (1.12-3.88) and 3.88 (0.96-5.97) RR 1.52 (1.49-4.57) and 1.94 (1.27-2.96), RD 23.16 (10.3-36.0) and 32.6 (15.7-49.6) for the TG and GG genotypes, suggesting a possible dominant effect of this polymorphism.

MDM2 genotypes and survival analysis

Follow-up of patients regarding survival was performed with median duration of 11.25 months (range, 0.5-127.5 months). A total of 84 patients suffered cancer related deaths with mean follow up time of 10.73 months and for the patients who survived the follow up period (censored patients), the follow-up time was 17.61 months. It was observed that the MDM2 309GG (Figure 2A) and GT+GG (Figure 2B) genotype was significantly associated with poor survival p<0.0001. Patient's survival was calculated using log-rank (Mantel–Cox) test. The estimated median survival time for patients with 309 TT, TG, GG+TG and GG genotype were 35.5, 13.5, 11.0 and 6.0 months respectively.

Discussion

In the present study, we examined whether genetic polymorphisms in MDM2 is associated with the risk of developing NSCLC. Our results obtained by analyzing 136 NSCLC patients and 136 controls demonstrate that the functional polymorphisms in the MDM2 promoter have a significant impact on the risk of developing NSCLC. The GG genotype was associated with the approximately 3.9 fold increased risk of NSCLC and was more predominant in subjects who were in advanced stage (p<0.01) with positive metastasis status at diagnosis (p<0.002). The frequency of GG allele was more among patients with squamous-cell carcinoma than adenocarcinoma of NSCLC. Patients of old age group (>45y) and females also showed higher frequency of risk allele (309GG). Higher frequency of GG genotype was found in patients who smoke cigarette as well as bidi as their smoking habit. Individuals with at least one 309T allele were at decreased risk of overall NSCLC as compared to those with 309GG genotype. Histologically poorly differentiated SCC and moderately differentiated ADC patients showed higher percentage of 309GG genotype.

The observations in this study are biologically plausible and consistent in several ways with previous studies. MDM2 negatively regulates the TP53 pathway that targets TP53 for degradation, and over expression or amplification of MDM2 has been frequently observed in many human cancer types, including lung cancer [13,15-18]. The high expression level of MDM2 is associated with the susceptibility to carcinogenesis. Over expression of mdm2 in the murine mammary epithelium resulted in mammary tumors [19], MDM2-transgenic mice developed tumors, produced an average of four fold more mdm2 in various tissues relative to non transgenic mice [20].

MDM2 has been shown to interact with several key tumor suppressors, including Rb [11], p53-family protein, p73 [21], the growth suppressor p14/p19 [22] and p53 [23]. Over expression of MDM2 and the subsequent expression of p21 are induced by p53 in response to DNA damage caused by exposure to environmental carcinogens, leading to an arrest of cell cycle, which in turn allows sufficient time for DNA repair [24]. Over expression of MDM2 was correlated with a

MDM2 G2580T Genotype	Cases (n=136)	Control (n=136)	OR*(95% CI)	RR**	RD***	P value
TT(ref)	28	58	1.00	1.00		
TG	78	62	2.60(1.49-4.57)	1.52(1.20-1.93)	23.16(10.3-36.0)	0.0009
GG	30	16	3.88(1.82-8.27)	1.94(1.27-2.96)	32.6(15.7-49.6)	0.0004
TG+GG	108	78	2.87(1.68-4.91)	1.61(1.29-2.01)	25.5(13.3-37.7)	0.0001

*OR- Odd ratio
**RR- Risk ratio
***RD- Risk difference

Table 3: Risk of lung cancer associated with the MDM2 (-309T/G) genotypes.

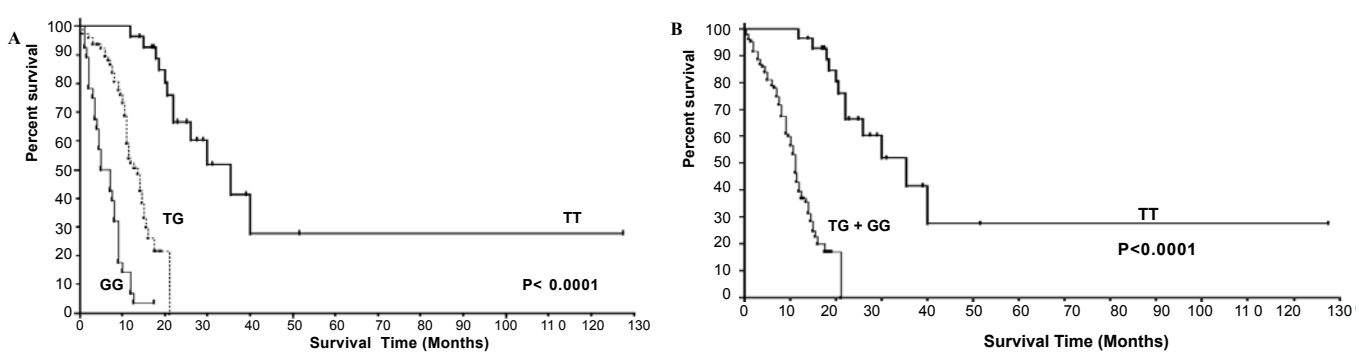

Figure 2: Kaplan-Meier survival curves for NSCLC patients with respect to MDM2 (309T/G) polymorphism.

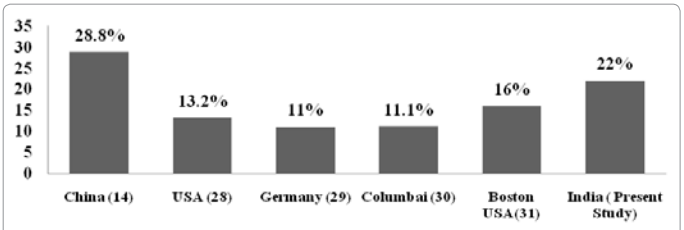

Figure 3: World scenario of MDM2 309GG genotype in lung cancer.

decreased level of p21 expression in epithelial breast cancer but with an increased level of p21 in oral squamous cell carcinoma.

MDM2G309T polymorphism occurs near the element believed to direct the intronic p53-response promoter activity of the gene, thus resulting in increased levels of MDM2 RNA and protein in an *in vitro* functional assay [12]. The G allele is correlated with a higher promoter activity which increases the binding affinity of Sp1 to the promoter of MDM2 [12], resulting in increased MDM2 expression which causes high rate of P53 protein degradation and may result in decreased apoptosis and ultimately the cancer. ºThus individuals carrying G allele may be at higher risk for developing NSCLC.

Various reports suggest an increased risk of lung cancer for the G allele of the MDM2 SNP309 in populations of various countries [14,25,26] (Figure 3). Our results were similar to the report of Zhang et al. [15].

Present study found that the risk was higher for squamous cell lung cancer may probably induced by tobacco smoke [27]. Significant death was observed in cells with the MDM2 TT genotype (20-35% of the total cell population) but not in cells with the MDM2 GG genotype (only 2-3% of the total cell population) after etoposide treatment to induce DNA damage, which activates the TP53 pathway and leads to DNA repair, cell cycle arrest, and apoptosis [12]. In addition MDM2 polymorphism was significantly associated with the poor survival outcome of patients with 308GG genotype and the median survival times were found to be of just 6 months. To the best of our knowledge, this is the first case-control study to investigate the polymorphism in cell cycle regulatory gene MDM2 (309T/G) and its association with NSCLC risk among Indian Patients [28-32].

Conclusion

In conclusion, present study shows that genetic polymorphism in cell cycle regulatory genes MDM2 contribute to the risk of developing NSCLC in Indian Patients. In addition 309GG genotype was associated with an increased risk than 309TT genotype. Study further suggest that 309T/G polymorphism in MDM2 gene might be a useful genetic marker in peripheral blood to determine susceptibility to advanced stage with distant metastasis status of NSCLC. However, because this is the first report concerning the MDM2 polymorphism and the risk of NSCLC in the Indian population, independent large population-based prospective studies for more rigorous analyses of subgroups are needed to validate our findings.

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References

1. Spitz MR, Wei Q, Dong Q, Amos CI, Wu X (2003) Genetic susceptibility to lung cancer: the role of DNA damage and repair. Cancer Epidemiol Biomarkers Prev 12: 689-698.
2. Banker DD (1955) J Post Grad Med 1: 108.
3. Nagrath SP, Hazra DK, Lahiri B, Kishore B, Kumar R (1970) Primary carcinoma of lung: clinico-pathological study of 35 cases. Indian J Chest Dis 12: 15-24.
4. Momand J, Zambetti GP (1997) Mdm-2: "big brother" of p53. J Cell Biochem 64: 343-352.
5. Momand J, Zambetti GP, Olson DC, George D, Levine AJ (1992) The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 69:1237-1245.
6. Chen J, Wu X, Lin J, Levine AJ (1996) Mdm-2 inhibits the G_1 arrest and apoptosis functions of the p53 tumor suppressor protein. Mol Cell Biol 16: 2445-2452.
7. Haupt Y, Barak Y, Oren M (1996) Cell type-specific inhibition of p53-mediated apoptosis by Mdm2. EMBO J 15: 1596-1606.
8. Martin K, Trouche D, Hagemeier C, Sorensen TS, La Thangue NB, et al. (1995) Stimulation of E2F1/DP1 transcriptional activity by MDM2 oncoprotein. Nature 375: 691-694.
9. Xiao ZX, Chen J, Levine AJ, Modjtahedi N, Xing J, et al. (1995) Interaction between the retinoblastoma protein and the oncoprotein MDM2. Nature 375: 694-698.
10. Brown DR, Thomas CA, Deb SP (1998) The human oncoprotein MDM2 arrests the cell cycle: elimination of its cell-cycle-inhibitory function induces tumorigenesis. EMBO J 17: 2513-2525.
11. Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B (1992) Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 358: 80-83.
12. Freedman DA, Levine AJ (1999) Regulation of the p53 protein by the MDM2 oncoprotein--thirty-eighth G.H.A. Clowes Memorial Award Lecture. Cancer Res 59: 1-7.
13. Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, et al. (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 119: 591-602.
14. Chiara M , Maria C, Gian L, Martina B, Roberta B, et al. (2006) Association between MDM2-SNP309 and age at colorectal cancer diagnosis according to p53 mutation status. J Natl Cancer Inst 98: 285-288.
15. Zhang X, Miao X, Guo Y, Tan W, Zhou Y, et al. (2006) Genetic polymorphisms in cell cycle regulatory genes MDM2 and TP53 are associated with susceptibility to lung cancer. Hum Mutat 27: 110-117.
16. Higashiyama M, Doi O, Kodama K, Yokouchi H, Kasugai T, et al. (1997) MDM2 gene amplification and expression in non-small-cell lung cancer: immunohistochemical expression of its protein is a favourable prognostic marker in patients without p53 protein accumulation. Br J Cancer 75: 1302-1308.
17. Momand J, Jung D, Wilczynski S, Niland J (1998) The MDM2 gene amplification database. Nucleic Acids Res 26: 3453-3459.
18. Eymin B, Gazzeri S, Brambilla C, Brambilla E (2002) Mdm2 overexpression and p14(ARF) inactivation are two mutually exclusive events in primary human lung tumors. Oncogene 21: 2750-2761.
19. Dworakowska D, Jassem E, Jassem J, Peters B, Dziadziuszko R, et al. (2004) MDM2 gene amplification: a new independent factor of adverse prognosis in non-small cell lung cancer (NSCLC). Lung Cancer 43: 285-295.
20. Lundgren K, Montes de Oca Luna R, McNeill YB, Emerick EP, Spencer B, et al. (1997) Targeted expression of MDM2 uncouples S phase from mitosis and inhibits mammary gland development independent of p53. Genes Dev 11: 714-725.
21. Jones SN, Hancock AR, Vogel H, Donehower LA, Bradley A (1998) A Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. Proc Natl Acad Sci U S A 95: 15608-15612.
22. Dobbelstein M, Wienzek S, Konig C, Roth J (1999) Inactivation of the p53-homologue p73 by the mdm2-oncoprotein. Oncogene 18: 2101-2106.
23. Zhang Y, Xiong Y, Yarbrough WG (1998) ARF promotes MDM2 degradation

and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell 92: 725-734.

24. Deb SP (2003) Cell cycle regulatory functions of the human oncoprotein MDM2. Mol Cancer Res 1: 1009-1016.

25. Powell BL, van Staveren IL, Roosken P, Grieu F, Berns EM, et al. (2002) Associations between common polymorphisms in TP53 and p21WAF1/Cip1 and phenotypic features of breast cancer. Carcinogenesis 23: 311-315.

26. Park SH, Choi JE, Kim EJ, Jang JS, Han HS, et al. (2006) MDM2 309T>G polymorphism and risk of lung cancer in a Korean population. Lung Cancer 54: 19-24.

27. Jun HJ, Park SH, Lee WK, Choi JE, Jang JS, et al. (2007) Combined effects of p73 and MDM2 polymorphisms on the risk of lung cancer. Mol Carcinog 46: 100-105.

28. Deutsch-Wenzel RP, Brune H, Grimmer G, Dettbarn G, Misfeld J (1983) Experimental studies in rat lungs on the carcinogenicity and dose-response relationships of eight frequently occurring environmental polycyclic aromatic hydrocarbons. J Natl Cancer Inst 71: 539-544.

29. Li G, Zhai X, Zhang Z, Chamberlain RM, Spitz MR, et al. (2006) MDM2 gene promoter polymorphisms and risk of lung cancer: a case-control analysis. Carcinogenesis 27: 2028-2033.

30. Mittelstrass K, Sauter W, Rosenberger A, Illig T, Timofeeva M, et al. (2008) Early onset lung cancer, cigarette smoking and the SNP309 of the murine double minute-2 (MDM2) gene. BMC Cancer 8: 113.

31. Pine SR, Mechanic LE, Bowman ED, Welsh JA, Chanock SC, et al. (2006) MDM2 SNP309 and SNP354 are not associated with lung cancer risk. Cancer Epidemiol Biomarkers Prev 15: 1559-1561.

32. Liu G, Wheatley-Price P, Zhou W, Park S, Heist RS, et al (2008) Genetic polymorphisms of MDM2, cumulative cigarette smoking and non small cell lung cancer risk. Int J Cancer 122: 915-918.