

Variation in the promoter region of the myeloperoxidase gene is not directly related to lung cancer risk among male smokers in Finland

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Abstract

In order to examine whether a polymorphism in the promoter region of the myeloperoxidase (MPO) gene is associated with lung cancer among male smokers, we conducted a case–control study nested within a Finnish clinical trial cohort. Although we found no evidence of an overall association between lung cancer risk and MPO genotype, the variant MPO genotype was associated with an increased risk of lung cancer among a subset of older men. These findings contrast with those from previous studies that report decreased lung cancer risk among MPO variant individuals. © 2001 Published by Elsevier Science Ireland Ltd.

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1. Introduction

Lung cancer is the leading cause of cancer death worldwide. In the US, lung cancer accounts for approximately 15% of all cancer cases, and 29% of all cancer deaths [1]. An overwhelming amount of evidence indicates that cigarette smoking is the major cause of lung cancer; in fact, it has been estimated that 85% of all lung cancer deaths are caused by smoking [2]. Despite the strong association between smoking and lung cancer, only a small fraction of smokers will get lung cancer during their life-

time, which suggests other environmental and genetic factors are important mediators of risk.

Nutritional status and occupational exposures to radon, asbestos, and organic and inorganic gases and vapors are well-recognized as environmental risk factors for lung cancer [2]. The list of genetic factors that may be associated with increased susceptibility to this malignancy includes specific polymorphisms in the genes encoding cytochrome P450 1A1 (CYP1A1), cytochrome P450 2E1 (CYP2E1), glutathione-S-transferase M1 (GSTM1), and myeloperoxidase (MPO) [3,4]. An evaluation of such factors may be useful in identifying high-risk segments of the population and developing more effective strategies for lung cancer detection and prevention.

MPO is an enzyme that occurs primarily in the

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lysosomes of neutrophils. MPO is thought to play an important role in neutrophil-mediated defense against invading microorganisms and may be involved in tumor immunosurveillance [5]. Under most circumstances, MPO-associated oxidants that are generated when MPO comes in contact with chloride ions and the hydrogen peroxide produced during neutrophil stimulation remain confined within phagosomes [6]. In the lung, however, leakage or secretion of MPO and hydrogen peroxide can occur after inhalation of various irritants, including particulate matter in cigarette smoke [7,8]. In experimental systems, it has been shown that MPO can catalyze the bioactivation of at least two classes of chemical carcinogens: polycyclic aromatic hydrocarbons (PAHs) and aromatic amines [9,10]. The putative role of MPO in the activation of tobacco PAHs forms the basis of the hypothesis that MPO status may mediate susceptibility to pulmonary carcinogenesis [4].

A single base substitution in an *Alu* repeat in the promoter region of the MPO gene has been shown to have functional significance *in vitro*. The presence of an A rather than a G, 463 bases upstream from the MPO gene, appears to decrease expression by destroying an SP1 transcription factor binding site [11]. Consistent with this finding, the G/G genotype has also been associated with higher MPO gene expression than the A/G genotype in primary myeloid leukemia cells [12]. Results from the first study to evaluate the relationship between MPO genotype and lung cancer suggested that individuals who inherit two copies of the A allele may be less susceptible to disease [4]. In that study, however, there was no evidence that the presence of a single variant allele decreased lung cancer risk, and the association between the MPO A/A genotype and decreased lung cancer risk was statistically significant only among Caucasians in the study population.

In order to examine further the association between MPO genotype and lung cancer, we conducted a case-control study nested within the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study. The ATBC study was a primary prevention trial designed to determine whether supplementation with alpha-tocopherol or beta-carotene could reduce the incidence of cancer among male smokers [13]. The size of this study, along with the availability of data on general characteristics of the study population

(including detailed dietary, smoking, and medical histories), a fasting blood sample obtained for each individual at baseline, and an active prospective follow-up to ascertain all cancer end-points, made this an ideal cohort for studying whether the genetic polymorphism in the promoter region of the MPO gene is associated with lung cancer among male smokers.

2. Materials and methods

2.1. Study population

The original study population consisted of male smokers participating in the ATBC study. During the period from 1985 through 1988, a total of 29 133 men in southwestern Finland, who were aged 50–69 years, and who smoked at least five cigarettes/day, were randomly assigned to receive either supplements or placebo. The overall design and rationale of this study have been published [13]. Men were excluded from the study if they had a history of cancer other than non-melanoma cancer of the skin or carcinoma *in situ*, severe angina upon exertion, chronic renal insufficiency, liver cirrhosis, chronic alcoholism, anti-coagulant therapy, other medical problems that might have limited long-term participation in the study, or the current use of dietary supplements containing vitamin E (>20 mg/day), vitamin A (>20 000 IU/day), or beta-carotene (>6 mg/day). The ATBC study was approved by the institutional review boards of the US National Cancer Institute and the National Public Health Institute of Finland.

2.2. Selection of cases and controls

A nested case-control sample set was constructed based on the availability of a whole blood sample collected between April 1992 and March 1993 from 20 305 men. Incident primary cases of lung cancer diagnosed on or before December 31, 1994 were identified through the Finnish Cancer Registry and the Register of Causes of Death. Cases having multiple cancers were excluded. Using incidence density sampling, controls were matched 1:1 to cases on age (± 5 years), intervention group, study clinic, and date of blood draw (± 45 days).

The medical records of the intervention cases (until

April 30, 1993) were reviewed independently by two study physicians, and follow-up cases (until December 31, 1994) were reviewed by one study physician. Histological specimens were available for 93% of the cases. The histological type of the primary lung tumor was determined from a review of specimens by two study pathologists for the intervention period cases, and from local hospital pathology reviews for the post-intervention cases. The final case–control sample used for epidemiological analysis consisted of 315 cases and 311 controls.

2.3. DNA isolation and MPO genotype analysis

DNA was isolated from whole blood samples as described previously [14]. The MPO polymorphism analysis was performed using a PCR-based approach. Probes with a predicted T_m near 68°C were selected, with the polymorphic base near the center. Flanking PCR primers were selected based on the penalty score calculated by PrimerExpress, a T_m of near 60°C, balanced length, and amplicon size. The oligonucleotide sequences for probes and primers to detect the polymorphic site at position –463 were: the forward primer 5'-CGGTATAGGCACACAATGGTGAG-3'; the reverse primer 5'-GGCCAGGCTGGTCTTGAA-C-3'; the G allele probe, VIC-AGTGATCCACC-GCCTCAGCCTCC-TAMRA; and the A allele probe, FAM-AAG TGATCCACTGCCTCAGCCT-CCA-TAMRA. The probes and primers were synthesized and purified by PE Biosystems (Foster City, CA). The reactions (10 μ l) contained 20 ng of genomic DNA, 1 \times TaqMan™ Master Mix (Roche Molecular Systems, Branchburg, NJ), dual-labeled probes (100 nM each), and PCR primers (900 nM each). The reactions were performed in 96-well MicroAmp Optical reaction plates with caps (PE Biosystems). The plates were incubated in PE Biosystems 9600 thermal cyclers at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 62°C for 1 min. The fluorescence was measured using the ABI Prism™ 7700 Sequence Detector (PE Biosystems) and analyzed with Sequence Detection System version 1.6.3 (PE Biosystems). Amplified DNA from several individuals of each genotype was analyzed by agarose gel electrophoresis to confirm amplicon size, and sequenced to confirm the genotype. A random sample of 10% of the specimens was assayed a second time,

and showed 100% concordance. Samples that produced a single fluorescence signal consistent with VIC emission were classified as G/G homozygotes; those that produced a single fluorescence signal consistent with FAM emission were classified as A/A homozygotes; and those that produced both fluorescence signals were classified as A/G heterozygotes.

2.4. Statistical analyses

The Chi-square test was used to test the null hypothesis that the distribution of MPO genotypes was the same for cases and controls. Conditional logistic regression was used to evaluate the association between MPO genotype and lung cancer incidence. For statistical modeling, the MPO genotype was characterized as G/G (reference category) or A/X (heterozygous and homozygous variants combined due to the low prevalence of the A/A genotype). Baseline covariates were identified as potential confounders by examining their distribution by case–control status and MPO genotype (Wilcoxon signed-rank test for continuous variables; Chi-square test for categorical variables). Selected baseline covariates were included in the multivariate model if they changed the odds ratio by more than 20% or caused a significant change in the likelihood ratio statistic ($P < 0.05$). Selected covariates were evaluated as effect modifiers by examining the change in the likelihood ratio statistic after including the covariate and the genotype-covariate cross-product term in the conditional logistic regression model. If a cross-product term was found to be statistically significant, an unmatched analysis (using unconditional logistic regression adjusted for the original matching criteria) stratified by the putative effect modifier was conducted. This was done to avoid the loss of subjects due to splitting of matched sets that fell into different strata defined by the effect modifier. Continuous covariates evaluated as effect modifiers were categorized into quartiles based upon their distribution among control subjects. The final conditional logistic regression model for main effects included the MPO genotype (G/G or A/X), along with the amount and duration of smoking (as continuous variables). All statistical analyses were conducted using STATA 6.0 (Stata Corporation, College Station, TX).

3. Results

Univariate analyses of baseline data on smoking, anthropometric, dietary, lifestyle, occupational, and medical history characteristics that might be related to lung cancer risk in this population revealed only a few differences between cases and controls. Due to the study matching, there were no case–control differences in age (the median baseline age was 60 years for cases and 59 years for controls) or intervention group distributions. Compared with controls, however, cases smoked more cigarettes/day ($P = 0.001$), smoked for a greater number of years ($P < 0.001$), and had a lower body mass index ($P = 0.02$). Independent of the differences in smoking, cases were also more likely to have had a history of chronic bronchitis ($OR = 1.78$, $P = 0.03$). Cases and controls did not appear to differ with regard to marital status, education, physical activity, asbestos exposure, or history of asthma or emphysema.

In this study, we found no direct association between MPO genotype and lung cancer risk (Table 1). Given the sample size of our study and the observed prevalence of the combined (A/G + A/A) genotype in our control population, we had adequate power ($>80\%$, $\alpha = 0.05$) to detect an OR of 0.6 or lower for lung cancer risk, for the combined genotype versus the G/G genotype. Furthermore, the distribution of MPO genotypes in our control population suggested that the alleles were in Hardy–Weinberg equilibrium: G/G = 66% (95% CI, 61–71%); G/A = 27% (95% CI, 22–32%); A/A = 7% (95% CI, 5–10%). This indicates the absence of genetic drift or any selective advantage for particular MPO alleles in this population.

When we evaluated potential gene–environment interactions, we found that the association between

MPO genotype and lung cancer risk was modified by both the age at randomization ($P = 0.007$ for the interaction term) and duration of smoking ($P = 0.014$ for the interaction term) as continuous variables. Analysis of the MPO genotype stratified by quartile of age (and adjusted for the amount and duration of smoking), showed that among men of 64 years and older, those with the A/G or A/A genotype were nearly three times more likely to develop lung cancer compared with those with the G/G genotype (Table 2). In contrast, a similar analysis stratified by duration of smoking (and adjusted for age) revealed no strong or statistically significant associations within any of the strata (data not shown). Interpretation of these results should be made with caution, however, since age and duration of smoking are highly correlated ($r = 0.66$) in this clinical trial cohort [15]. We found no evidence that alpha-tocopherol or beta-carotene supplementation modified the association between MPO genotype and lung cancer in this population.

4. Discussion

In the present study, we found no evidence of a direct association between MPO genotype and lung cancer risk in male smokers. We did, however, observe an increased risk associated with the variant allele in men of >64 years old. Our results are consistent with the results of previous case–control analyses from the ATBC study which failed to demonstrate direct associations between lung cancer risk and genetic polymorphisms in CYP1A1 and GSTM1, two other genes implicated in pulmonary PAH activation (Ratnasinghe et al., in preparation) [15].

To our knowledge, this is the fifth study to examine

Table 1
Risk of lung cancer by MPO genotype

	Cases (%)	Controls (%)	OR (95% CI) ^a	OR (95% CI) ^b
G/G	191 (60.6)	206 (66.2)	1.00	1.00
A/G	108 (34.3)	84 (27.0)	1.36 (0.96–1.92)	1.21 (0.84–1.75)
A/A	16 (5.1)	21 (6.8)	0.80 (0.39–1.66)	0.72 (0.32–1.65)
A/X (A/G + A/A)	124 (39.4)	105 (33.8)	1.27 (0.91–1.76)	1.13 (0.80–1.61)

^a Unadjusted.

^b Adjusted for years smoked, and number of cigarettes/day.

Table 2

The association between MPO genotype and lung cancer risk, stratified by age at randomization

Age (years)	G/G		A/X		
	OR (reference)	Cases/controls	OR (95% CI) ^a	OR (95%CI) ^b	Cases/controls
50–55	1.00	51/46	0.85 (0.46–1.58)	0.82 (0.43–1.54)	34/36
56–59	1.00	45/56	0.93 (0.48–1.80)	0.95 (0.47–1.90)	24/32
60–63	1.00	62/56	1.44 (0.74–2.81)	1.29 (0.65–2.57)	32/20
64–69	1.00	33/48	2.91 (1.40–6.05) ^c	2.92 (1.33–6.43) ^d	34/17

^a Unadjusted.^b Adjusted for intervention group, years smoked, and number of cigarettes per day.^c $P = 0.004$.^d $P = 0.008$.

the relationship between MPO genotype and lung cancer risk. Although the four previous studies found material associations between MPO genotype and lung cancer risk, estimates of the nature and extent of the association varied considerably between studies [4,16–18]. In a hospital-based, case–control study conducted in Los Angeles County [4], no difference in lung cancer risk was observed between Caucasians with the G/G versus the A/G genotype; however, Caucasians with the A/A genotype were less likely to have lung cancer than Caucasians who had one or two G alleles ($OR = 0.26$; 95% CI, 0.07–0.75). Among African–Americans in the same study, the protective association between the A/A genotype and lung cancer risk was much less pronounced ($OR = 0.61$; 95% CI, 0.26–1.41) and was not statistically significant. Similar results were obtained from a recent, population-based, case–control study conducted in Hawaii [17]. In that study, little difference in lung cancer risk was observed between individuals with the G/G versus the A/G genotype ($OR = 0.71$; 95% CI, 0.42–1.21), and there was limited, although not statistically significant, evidence that the A/A genotype was protective ($OR = 0.51$; 95% CI, 0.17–1.4). In contrast, in a hospital-based, case–control study conducted in Berlin, Germany [16], individuals with the A/G genotype were less likely ($OR = 0.54$; 95% CI, 0.34–0.86) to have lung cancer than G/G individuals, whereas a slight increase in risk was observed between individuals with the A/A genotype versus those who had one or two G alleles ($OR = 1.24$; 95% CI, 0.29–6.13).

Although the prevalence of the variant MPO allele was similar across studies, discrepancies in results

may be related to differences in the level of diversity among the study populations with regard to gender and/or environmental exposures. All of the previous studies included smoking and non-smoking men and women, whereas our study population was comprised exclusively of male smokers. Evidence that sex-specific differences in the study populations might be biologically important comes from recent analyses of the association between MPO genotype and multiple sclerosis, and MPO genotype and Alzheimer's disease [19,20]. In a study of multiple sclerosis patients, the G/G genotype was associated with early onset disease in females, but not in males [19]. In a study of patients with Alzheimer's disease, 73% of females were G/G, whereas only 48% of males were G/G. In that study, the prevalence estimate for the G/G genotype in the unaffected population was near 60% for both males and females [20]. For both diseases, the biological basis of the apparent sex-related effect modification is poorly understood.

Since individuals in the ATBC study population were all chronic smokers, we may have been limited in our ability to distinguish differences in lung cancer risk according to genotype, if such differences were related to the amount/duration of smoking. Numerous enzymes are known to be involved in the bioactivation of tobacco carcinogens. It is conceivable that at high levels of smoke exposure, the induction of other enzymes may obscure the contribution that MPO might make toward carcinogen activation in the lung.

Alternatively, differences between the findings from the various studies may be related to geographic variables. It is known that environmental ozone exposure can lead to neutrophil accumulation in human

lungs [21,22] and that pulmonary MPO activity is directly related to the extent of neutrophil infiltration. Unusually high ozone concentrations in the Los Angeles area, for example, may partially explain why the MPO genotype is more strongly associated with lung cancer in this geographic region than in another [17].

In our study, the genotype associated with the highest level of MPO gene expression (G/G) appeared to be protective among the oldest quartile of men. This finding lends indirect support to the hypothesis that MPO may be involved in tumor cell cytotoxicity. In fact, an association between an increased incidence of malignancy and congenital MPO deficiency has been documented in numerous reports [5]. Among the controls in the present study, the proportion of men with the G/G genotype increased with age. Although direct evidence for such a mechanism is lacking, it is tempting to speculate that an age-related decline of primary tumor immunosurveillance activity might lead to an increased dependence on neutrophil-mediated, anti-tumor defense mechanisms, giving individuals with the G/G genotype a survival advantage. In other words, high levels of MPO-mediated tumoricidal activity may help smokers reach older ages without getting lung cancer. It is also possible that the source of our study population, relatively healthy middle-aged smokers who were willing and able to participate in a long-term prevention trial, may have contributed to this apparent age association. If having one or two A alleles is associated with an increased risk of other comorbidities (especially those related to smoking) that manifest prior to middle-age, there may have been an under-representation of older men with the A allele who were eligible to participate in the trial population from which our study sample was derived.

Various lines of evidence suggest that the histological type of a lung tumor may be determined by the particular initiating agent to which an individual is exposed [17,23–25]. It is therefore conceivable that MPO (which has been associated with the activation of PAHs, but not nitrosamines) might play a differential role in the development of pulmonary squamous cell carcinomas that have been associated with PAH exposure, versus adenocarcinomas which have been linked to nitrosamine exposure. In our study, 140 (44%) lung cancer cases were squamous cell carcinoma,

49 (15.5%) were small cell carcinomas, and 55 (17.5%) were adenocarcinomas. The relatively small sample represented within strata defined by these tumor types resulted in unstable estimates of risk and limited any inferences regarding such associations.

In the present study, we found no evidence of a direct association between lung cancer risk and a specific polymorphism in the MPO gene among white male smokers. These findings contrast with those from previous studies that reported a decreased lung cancer risk among MPO variant individuals, suggesting that further work is needed in order to determine the extent to which MPO genotype mediates lung cancer susceptibility.

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References

- [1] C.C. Boring, T.S. Squires, T. Tong, *Cancer Statistics* 1994, *CA Cancer J. Clin.* 44 (1994) 7–26.
- [2] J.H. Lubin, Risks for major cancers: lung and larynx, in: A. Hurray (Ed.), *Cancer Rates and Risks*, NIH Pub. No. 96-691, National Cancer Institute, Bethesda, MD, 1996, pp. 158–162.
- [3] L. Le Marchand, L. Sivaraman, L. Pierce, A. Seifried, A. Lum, L.R. Wilkens, A. Lau, Associations of CYP1A1, GSTM1, and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens, *Cancer Res.* 58 (1998) 4858–4863.
- [4] S.J. London, T.A. Lehman, J.A. Taylor, Myeloperoxidase genetic polymorphism and lung cancer risk, *Cancer Res.* 5 (1997) 5001–5003.
- [5] F. Lanza, Clinical manifestation of myeloperoxidase deficiency, *J. Mol. Med.* 76 (1998) 676–681.
- [6] S.J. Klebanoff, Myeloperoxidase, *Proc. Assoc. Am. Physicians* 111 (1999) 383–389.
- [7] G.W. Hunninghake, R.G. Crystal, Cigarette smoking and lung destruction: accumulation of neutrophils in the lungs of cigarette smokers, *Am. Rev. Respir. Dis.* 128 (1990) 833–838.
- [8] B. Schmekel, S.E. Karlsson, M. Linden, C. Sundstrom, H. Tegner, P. Venge, Myeloperoxidase in human lung lavage.

- I. A marker of local neutrophil activity, *Inflammation* 14 (1990) 447–454.
- [9] W.G. Mallet, D.R. Mosebrook, M.A. Trush, Activation of (\pm)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene to diolepoxides by human polymorphonuclear leukocytes or myeloperoxidase, *Carcinogenesis* 12 (1991) 521–524.
- [10] S.J. Culp, D.W. Roberts, G. Talaska, N.P. Lang, P.P. Fu, J.O. Lay Jr., C.H. Teitel, J.E. Snawder, L.S. Von Tungeln, F.F. Kadlubar, Immunochemical 32P-postlabeling, and GC/MS detection of 4-aminobiphenyl-DNA adducts in human peripheral lung in relation to metabolic activation pathways involving pulmonary *N*-oxidation, conjugation, and peroxidation, *Mutat. Res.* 378 (1997) 97–112.
- [11] F.J. Piedrafita, R.B. Molander, G. Vansant, E.A. Orlova, M. Pfahl, W.F. Reynolds, An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element, *J. Biol. Chem.* 271 (1996) 14412–14420.
- [12] W.F. Reynolds, E. Chang, D. Douer, E.D. Ball, V. Kanda, An allelic association implicates myeloperoxidase in the etiology of acute promyelocytic leukemia, *Blood* 90 (1997) 2730–2737.
- [13] ATBC Cancer Prevention Study Group, The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers, *N. Engl. J. Med.* 330 (1994) 1029–1035.
- [14] K. Woodson, D. Ratnasinghe, N.K. Bhat, C. Stewart, J.A. Tangrea, T.J. Hartman, R. Stolzenberg-Solomon, J. Virtamo, P.R. Taylor, D. Albanes, Prevalence of disease-related polymorphisms among participants in a large cancer prevention trial, *Eur. J. Cancer Prev.* 8 (1999) 441–447.
- [15] K. Woodson, C. Stewart, M. Barrett, N.K. Bhat, J. Virtamo, D. Taylor, Effect of vitamin intervention on the relationship between GSTM1, smoking, and lung cancer risk among male smokers, *Cancer Epidemiol Biomarkers Prev.* 8 (1999) 965–970.
- [16] I. Cascorbi, S. Henning, J. Brockmoller, J. Gephart, C. Meisel, J.M. Muller, R. Loddenkemper, I. Roots, Substantially reduced risk of cancer of the aerodigestive tract in subjects with variant –463A of the myeloperoxidase gene, *Cancer Res.* 60 (2000) 644–649.
- [17] L. Le Marchand, A. Seifried, A. Lum, L.R. Wilkens, Association of the myeloperoxidase –463G to A polymorphism with lung cancer risk, *Cancer Epidemiol. Biomarkers Prev.* 9 (2000) 181–184.
- [18] M.B. Schabath, M.R. Spitz, X. Zhang, G.L. Delclos, X. Wu, Genetic variants of myeloperoxidase and lung cancer risk, *Carcinogenesis* 21 (2000) 1163–1166.
- [19] R.M. Nagra, B. Becher, W.W. Tourtellotte, J.P. Antel, D. Gold, T. Paladino, R.A. Smith, J.R. Nelson, W.F. Reynolds, Immunohistochemical and genetic evidence of myeloperoxidase involvement in multiple sclerosis, *J. Neuroimmunol.* 78 (1997) 97–107.
- [20] W.F. Reynolds, J. Rhee, D. Maciejewski, T. Paladino, H. Sieburg, R. Maki, E. Masliah, Myeloperoxidase polymorphism is associated with gender specific risk for Alzheimer's disease, *Exp. Neurol.* 155 (1999) 31–41.
- [21] D.E. Graham, H.S. Koren, Biomarkers of inflammation in ozone-exposed humans: comparison of the nasal and bronchoalveolar lavage, *Am. Rev. Respir. Dis.* 142 (1990) 152–156.
- [22] L. Calderon-Garciduenas, A. Rodriguez-Alcaraz, R. Garcia, G. Sanchez, G. Barragan, R. Camacho, L. Ramirez, Human nasal mucosal changes after exposure to urban pollution, *Environ. Health Perspect.* 102 (1994) 1074–1080.
- [23] M.S. Greenblatt, W.P. Bennett, M. Hollstein, C.C. Harris, Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis, *Cancer Res.* 54 (1994) 4855–4878.
- [24] M.F. Denissenko, A. Pao, M.-S. Tang, G.P. Pfeifer, Preferential formation of benzo(a) pyrene adducts at lung cancer mutational hotspots in p53, *Science* 274 (1996) 430–432.
- [25] D. Hoffmann, A. Rivenson, S.E. Murphy, F.-L. Chung, S. Amin, S.S. Hecht, Cigarette smoking and adenocarcinoma of the lung: the relevance of nicotine-derived nitrosamines, *J. Smoking Relat. Disord.* 4 (1993) 165–190.