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COMMENTARY

Air pollution and cancer: biomarker studies in human populations[†]

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Large cohort studies in the U.S. and in Europe suggest that air pollution may increase lung cancer risk. Biomarkers can be useful to understand the mechanisms and to characterize high-risk groups. Here we describe biomarkers of exposure, in particular DNA adducts as well as markers of early damage, including mutagenicity, other endpoints of genotoxicity and molecular biomarkers of cancer. Several studies found an association between external measures of exposure to air pollution and increased levels of DNA adducts, with an apparent levelling-off of the dose–response relationship. Also, numerous experimental studies *in vitro* and *in vivo* have provided unambiguous evidence for genotoxicity of air pollution. In addition, due to the organic extracts of particulate matter [especially various polycyclic aromatic hydrocarbon (PAH) compounds], particulate air pollution induces oxidative damage to DNA. The experimental work, combined with the data on frequent oxidative DNA damage in lymphocytes in people exposed to urban air pollution, suggests 8-oxo-dG as one of the important promutagenic lesions. Lung cancer develops through a series of progressive pathological changes occurring in the respiratory epithelium. Molecular alterations such as loss of heterozygosity, gene mutations and aberrant gene promoter methylation have emerged as potentially promising molecular biomarkers of lung carcinogenesis. Data from such studies relevant for emissions rich in PAHs are also summarized, although the exposure circumstances are not directly relevant to outdoor air pollution, in order to shed light on potential mechanisms of air pollution-related carcinogenesis.

Introduction

Results from prospective studies suggest that air pollution is likely to increase the risk of lung cancer. In the United States, results have been published from the Adventist Health Study on SMOG (1,2), based on 6338 California Seventh Day Adventists followed from 1977 through 1992, the Harvard Six Cities Study (3), based on 8111 residents of six US cities, followed from 1974 through 1989, and the American Cancer Society Study [ACS-II, (4)], based on the mortality experience of ~500 000 adult men and women who were followed from 1982 through 1998. All such studies suggest an increase in lung cancer risk in association with exposure to urban air pollutants, particularly PM10 or PM2.5. For research purposes, PM (particulate matter) is usually subdivided into PM10 (inhalable particles), PM2.5 (fine particles) and PM0.1 (ultrafine particles). These PM size cuts generally represent different sources and display different physical and chemical properties, but the physico–chemical characteristics responsible for PM-associated toxicity are only incompletely understood. The first published European cohort study examining long-term exposure to air pollution was conducted in the Netherlands (5), based on 120 852 adults living in 204 small towns and large cities throughout the Netherlands, and a second European study has been reported from Norway (6), where Nafstad and co-workers studied lung cancer incidence among 16 209 men living in Oslo, who were recruited in 1972–1973. Also in the European studies an increased risk ratio for lung cancer of ~1.10 for an increment of 10 $\mu g/m^3$ of NO_2 was found.

Epidemiological studies are extremely valuable, but their contribution can be supported and integrated by studies on biomarkers. Biomarkers have been introduced in chronic disease epidemiology under the assumption that they could improve the investigation of health effects of air pollution and other exposures, by (i) improving exposure assessment, (ii) increasing the understanding of mechanisms, e.g. by measuring intermediate biomarkers, and (iii) allowing the investigation of individual susceptibility.

Here we will describe biomarkers of exposure, in particular DNA adducts as well as markers of early damage, including mutagenicity, other genotoxic effects and molecular biomarkers of cancer. The discussion on the latter ones is focused on gene mutations and epigenetic changes. We consider not only direct evidence concerning biomarkers related to outdoor air pollution, but also evidence on other sources of compounds present in polluted air. In particular, tobacco smoke and indoor emissions from use of smoky coal fuel will be discussed as sources of polycyclic aromatic hydrocarbons (PAHs) and

Abbreviations: B(*a*)P, benzo[*a*]pyrene; PAH, polycyclic aromatic hydrocarbons.

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pollutants present in indoor air. Although not all exposure circumstances discussed are directly relevant to outdoor air pollution, biomarker data from such studies are highly valuable in shedding light on common mechanisms.

Biomarkers of exposure

DNA adducts and exposure to air pollution

A number of studies have considered DNA damage as an endpoint for the effects of air pollution, in particular 'bulky' DNA adducts, which are related to exposure to aromatic compounds, including PAH.

A systematic review was performed to evaluate whether metabolites of pyrene and DNA adducts are valid markers of low level environmental exposure to PAHs (7). Thirty five studies, with >10 subjects, that evaluated environmental air pollution to PAHs in relation to metabolites of PAHs, PAH–DNA adducts or protein adducts were identified. PAH metabolites and, to a less extent, PAH–DNA adducts correlated well at the group level with exposure to benzo[*a*]pyrene [B(*a*)P], even at low levels of air pollution.

In fact, as Table I suggests, studies in different countries have shown that the levels of WBC–DNA adducts were higher among subjects more heavily exposed to air pollutants. This observation has been made in different population categories, such as among police officers in Italy and Thailand (8,9), in residents in highly industrialized areas in Poland (10) and among bus drivers in Denmark (11). In all these cases the more exposed subjects had significant differences from those who were less exposed (see Table I).

More recently, a group of 114 workers exposed to traffic pollution and a random sample of 100 residents were studied in Florence. DNA bulky adducts were analysed in peripheral leukocytes donated at enrolment, by using ^{32}P-post-labeling. Adduct levels were significantly higher for traffic workers among never-smokers ($P = 0.03$) and light current smokers ($P = 0.003$). In both groups, urban residents tended to show higher levels than those living in suburban areas, and a seasonal trend emerged with adduct levels being highest in summer and lowest in winter (12).

In a study in Greece, the levels of bulky DNA adducts were measured by ^{32}P-post-labelling in lymphocytes of 194 non-smoking students living in the city of Athens and in the region of Halkida. Personal exposures to PAHs were significantly higher in Athens subjects. However, the highest adduct levels were observed in a subgroup of subjects living in Halkida, with a minimal burden of urban air pollution. Among the Halkida subjects (but not the remaining subjects) positive correlations were observed between DNA adducts and measured personal exposures to chrysene or B(*a*)P. A much clearer association of adducts with environmental tobacco smoke was observed (13).

In Denmark, Sorensen *et al.* (14) measured personal PM2.5 and black smoke exposure in 50 students four times over 1 year and analysed biomarkers of DNA damage. Personal PM2.5 exposure was found to predict 8-oxo-dG in lymphocyte DNA with an 11% increase in 8-oxo-dG/10 $\mu g/m^3$ increase in PM exposure ($P = 0.007$).

A case–control study nested in a large prospective study (EPIC) has been completed in Europe (15). Cases included newly diagnosed lung cancer ($N = 115$), upper respiratory cancers (pharynx, larynx) ($N = 82$), bladder cancer ($N = 124$), leukemia ($N = 166$) and COPD or emphysema deaths ($N = 77$), accrued after a median follow-up of 7 years among the EPIC ex-smokers and never-smokers. Leukocyte DNA adducts were analysed blindly using the nuclease P1 modification of the ^{32}P DNA post-labelling technique. The intensity of adduct patterns was generally stronger in the chromatograms of healthy non-smokers who developed a lung cancer in the following years in comparison with the other samples. The observed adduct profile has been previously described among subjects environmentally exposed to air pollution. Adducts

Table I. Studies on DNA or protein adducts in human populations exposed to different air pollution levels

Reference	Country	Population	Measure	Levels[a]	*P*-value	Notes
DNA adducts						
Perera *et al.* (10)	Poland	Highly-industrialized area	PAH–DNA adducts	$30.4/10^8$ versus 11 (rural area)	$P < 0.05$	Winter levels
Peluso *et al.* (8)	Italy	Police officers	Bulky DNA adducts	$1.3/10^8$ versus 0.9	<0.05	In summer: 2.8 versus 0.8 ($P < 0.001$)
Nielsen *et al.* (11)	Denmark	Bus drivers	PAH–DNA adducts	1.2 fmol/microg versus 0.585	0.04	Rural controls: 0.074, $P < 0.001$)
Palli *et al.* (12)	Italy	Traffic workers	Bulky DNA adducts	$13.7/10^9$ versus 11.0	0.10	Among never-smokers $P = 0.03$
Georgiadis *et al.* (13)	Greece	Students with different air pollution exposures	Bulky DNA adducts	1.25/108	<0.001 versus 1.54[b]	
Ruchirawa *et al.* (9)	Thailand	Police officers	Bulky DNA adducts	$1.6/10^8$ versus 1.2	0.03	
Sorensen *et al.* (14)	Denmark	Students with different pollution exposures	8-oxo-dG	0.01[c]	0.007	
Peluso *et al.* (15,123)	10 European countries	Residents with different pollution levels	Bulky DNA adducts	0.066[d]	0.0095	Never or ex-smokers
Protein adducts						
Pastorelli *et al.* (20)	Italy	Newspaper vendors	Benzopyrene–hemoglobin adducts	0.3 fmol/mg versus <0.1	0.09	
Richter *et al.* (21)	Germany	Children in towns with different pollution levels	4-ABP–hemoglobin adducts	30.7 pg/g Hb 20.7	<0.001	

[a] More exposed vs less exposed.

[b] Higher adducts levels in the least polluted area.

[c] Regression coefficient between unit increments of PM2.5 and adduct levels.

[d] Regression coefficient between unit increments of O_3 and adduct levels.

were associated with the subsequent risk of lung cancer, with an odds ratio (OR) of 1.86 (95% CI 0.88–3.93). The association with lung cancer was stronger in never-smokers (OR = 4.04; CI 1.06–15.42) and among the younger age groups. After exclusion of the 36 months preceding lung cancer onset the OR was 4.16 (1.24–13.88). Besides, the authors found an association of adduct levels with O_3, suggesting a possible role for photochemical smog in determining DNA damage of non-smokers in western Europe. This is consistent with the previous investigation in Florence, showing a significant relationship between cumulative O_3 exposure and bulky DNA adducts among non-smokers (12). O_3 is a marker of photochemical smog, produced by a complex series of reactions involving hydrocarbons and nitrogen dioxide, emitted primarily during combustion of fossil fuels by industry and transportation activities, and driven by ultraviolet (UV) radiation in sunlight. O_3 may have biological effects directly and/or via free radicals reacting with other air pollutants. After UV activation, PAHs may produce covalent adducts, e.g. benzo(*a*)anthracene, B(*a*)P and 1-hydroxypyrene DNA adducts (16,17). UV irradiation has been also shown to synergize with B(*a*)P to significantly enhance the expression levels of the tumour suppressor gene P53 (18). Recently, an enhancement of the signature of mutations produced by B(*a*)P, i.e. G→T + C→A transversions, has been found after UV irradiation (19).

Protein adducts

In a study among newspaper vendors, Pastorelli *et al*. (20) have found a higher level of benzopyrene–hemoglobin adducts, but the difference with less exposed populations was not statistically significant. Richter *et al*. (21) studied haemoglobin adducts formed by aromatic amines, including 4-aminobiphenyl, in groups of children. They found that children living in the most polluted city had significantly higher levels of adducts than those living in less polluted cities.

Dose–response relationship

Lewtas *et al*. (22) have observed that human populations exposed to PAHs via air pollution show a non-linear relationship between levels of exposure and WBC–DNA adducts. Among highly exposed subjects, the DNA adduct level per unit of exposure was significantly lower than the level measured at environmental exposures. The observation was confirmed in a meta-analysis of the epidemiological studies (23) (Figure 1). The same exposure-dose non-linearity was observed in lung DNA from rats exposed to PAH (22). One interpretation proposed for such observations is that saturation of metabolic enzymes or induction of DNA repair processes occur at high levels of exposure.

Biomarkers of early effect and disease

Genotoxicity of air pollution in experimental systems

Bacterial mutagenic activities of outdoor air pollution from anthropogenic combustion-related sources or its main components have been shown in a broad set of *in vitro* assays, as recently reviewed (24). Such data have revealed that the various PAH compounds present in virtually all combustion-related complex mixtures constitute an important source of genotoxicity. They may not however represent the sole or even the predominant class of mutagens present in outdoor air pollution, since mutagenicity of airborn particulate organics is caused by at least 500 mutagenic components of varying chemical classes (24). Also other factors, such as particle size and chemical reactions in the atmosphere, are known to affect the genotoxicity of ambient air (24). For instance, extractable PM, carcinogenic PAHs, and genotoxicity of environmental air pollution found in winter samples seem in many studies

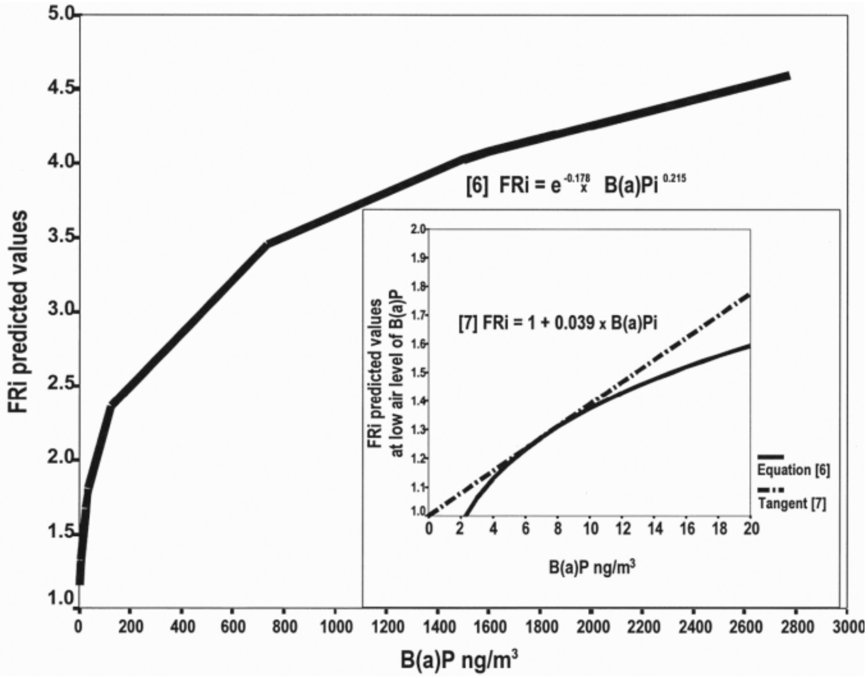

Fig. 1. Dose–response relation between frequency ratios and external B(*a*)P concentrations in work environments in a meta-analysis of occupational exposure to air pollution, as predicted from Equation 6. The inset shows an extrapolated dose–response curve at low exposure doses, assuming a linear dose–response relation, for B(*a*)P levels between 0 and 4.5 ng/m^3, the lowest value in the database. FR$_i$, frequency ratio for the *i*-th study [from (23)].

exceeding those detected in summer samples (7,25–28). Interestingly though, the seasonal trends observed in human studies have indicated that the levels of DNA adducts tend to be the highest in summer and the lowest in winter, as described in the previous sections.

Experimental work carried out *in vitro* and *in vivo* since the late 1970s have repeatedly shown lung toxicity, inflammatory effects, genotoxicity and rodent carcinogenicity of various types of particulate air pollution. Such effects have been especially reported not only for PM from diesel exhausts but also for urban air particulates (24,29–44). There are abundant data from cell free systems and cell culture experiments showing the capacity of various types of particulates, including diesel exhaust and urban particles, to cause oxidative DNA damage, mainly single strand breaks and 8-oxo-dG (8-oxo-7,8-dihydro-2′-deoxyguanosine) (reviewed in 45). In line with these data, *in vivo* experiments have demonstrated that diesel exhaust particles induce oxidative DNA damage in lung tissue in rodents, starting from low dose levels [e.g. (37,45–51)].

In rodent transgenic assays, both positive and negative results have been reported for induction of mutations in the transgene in lung tissue (37,43,52). Recently, transplacental exposure to diesel exhaust particles was found to induce deletions of the p^{un} allele in mice (53). In lung tumours induced by diesel exhaust in rats, K-*ras* and *p53* gene mutations were not common (54,55), but a high rate of K-*ras* mutations was observed in lung adenomas and adenocarcinomas induced following diesel exhaust exposure via intratracheal instillation (56). The animal gene mutation studies are summarized briefly in Table II.

A recent study reported germline mutagenicity of outdoor air pollution (57). Laboratory mice, housed for 10 weeks outdoors in an area with air pollution, exhibited an increased mutation rate at repetitive (expanded simple tandem repeat) DNA loci in the offspring. The mutation rate was reduced by 50% in those animals for whom the air was filtered through a high-efficiency particulate-air filter capable of removing practically all ($>$99%) particles $>$0.1 μm in diameter (57). The study thus suggests that air pollution, i.e. mutagens bound to the particles and/or particles themselves, is capable of causing heritable mutations, with a predominant effect on male germline (57).

A previous study indicated a 1.5- to 2.0-fold increase in germline mutation rate at the same repetitive loci in laboratory mice kept at an urban-industrial site but the experimental setting did not allow identification of the causative agents or fractions (58). Elevated germline mutation rates have also been seen in birds near industrial areas (59–61). However, mechanisms responsible for the observed germline mutagenesis in mice may be multiple, and the results should be interpreted with caution (62).

Biomarker studies on mutagenicity and cytogenetic effects in humans

Urinary mutagenicity was elevated in the *Salmonella* assay among non-smoking bus drivers exposed to polluted urban air, mainly traffic exhausts, as compared with mail carriers working in the same city (63). In addition, several but not all studies investigating cytogenetic effects (chromosome aberrations, micronuclei and sister chromatid exchange) in groups of healthy individuals from a wide variety of geographical locations with variable air pollution have reported positive findings, especially among traffic policemen (63–70).

Biomarker studies investigating *HPRT* gene mutations in healthy adults in association with air pollution did not find increased frequencies in peripheral blood lymphocytes (71,72). However, somatic *HPRT* mutation frequencies and aromatic DNA adducts were found to be correlated in cord blood samples from newborns of mothers living in polluted area in Poland, thus suggesting DNA damage *in uter*o (73) (Table II). *HPRT* mutations and DNA adducts were not correlated in peripheral lymphocytes of the mothers (73).

Mutations in lung cancer

In the next sections, we discuss some biomarker studies where the exposure circumstances are not directly relevant to oudoor air pollution. However, we suppose that biomarker data in association with sources of indoor air exposure to e.g. PAHs shed light on potential mechanisms of air pollution-related carcinogenesis.

The spectra of the *TP53* mutations occurring in human cancers has been widely used as a molecular biomarker in search for etiological factors involved in carcinogenesis (74–78). An array of scientific evidence has demonstrated associations between mutations of the *TP53* gene in lung tumours and exposure to tobacco smoke, with a unique PAH-related mutation spectrum, as extensively reviewed (76–79). The data show that *TP53* mutations occur more frequently in lung cancer from smokers than that from never-smokers, and that the frequency of *TP53* mutations is dependent on the daily amount of smoking (77,80). Mutations of the *TP53* gene, among other molecular changes, have also been found in pre-neoplastic lesions and normal-appearing tissue in the lungs of smokers (81–87). Such observations are not limited to lung cancer from smokers but there are data suggesting that lung cancer from never-smokers regularly exposed to second-hand smoke, a significant indoor air pollutant, also carries similar types of molecular alterations (86,87).

Lung cancer associated with indoor exposure to emissions from smoky coal combustion

Despite the overwhelming data accumulating on molecular features of smokers' lung cancer, literature data on lung cancer from non-smokers with other types of exposures relevant for air pollution are scanty. *TP53* mutations and *KRAS* mutations were investigated in lung tumours from non-smokers in a region in China, where households had for a long time been using smoky coal as fuel in unvented firepits or stoves (88). The study found a very high mutation frequency (71%) of *TP53* gene mutations in lung cancers from those exposed to emissions from smoky coal combustion. The mutations observed followed the spectrum typical of complex mixtures rich in PAHs (89), with 76% being GC to TA transversions and 100% of the guanines involved on the non-transcribed strand (88). *KRAS* mutations in the lung tumours were lower in frequency (29%), but they were almost entirely GC to TA transversions (86%) (88).

A follow-up of the study, involving 102 lung cancers from non-smoking women exposed to unvented coal smoke in Xaun Wei county, showed very similar findings, with frequencies of 21.9 and 66.7% for GC to TA transversions for *TP53* and *KRAS* genes, respectively (90). Interestingly, the frequency and type of *KRAS* mutations among these non-smoking women were comparable with those found among smoking men from Xuan Wei and other regions in China where natural gas is used as the main domestic fuel (90). Recently, sputum

Table II. Summary of findings from human biomarker studies and animal *in vivo* studies on gene mutations and gene promoter hypermethylation associated with exposure to indoor or outdoor air pollution, some of their major components, or cigarette smoke (as a model exposure for PAHs)

Alteration/molecular biomarker studied	Cell type/tissue studied	Type of exposure associated	Comments	Reference
Gene mutations				
Human studies				
HPRT gene mutations in healthy newborns	Cord blood lymphocytes	Polluted outdoor air (urban)	Mutation frequency correlated with presence of aromatic DNA adducts. Negative findings in the mothers	(73)
			Negative findings in peripheral blood lymphocytes in adults	(71,72)
TP53 gene and/or *RAS* gene mutations in non-lung cancer patients who are smokers, or non-smokers without evidence of lung cancer	Lung tumour tissue; non-malignant epithelial cells from sputum	PAH-rich emissions from smoky coal combustion in unvented fireplaces or stoves (indoor exposure)	Frequent in lung tumour tissue. In non-malignant cells, *TP53* mutations present with a low frequency	(88,90,91)
Experimental studies				
Mutations of the *lambda*/*lacI* transgene in rat transgenic assay	Lung tissue	Diesel exhaust (inhalation exposure, 4 weeks)	Also ^{32}P-labelled aromatic DNA adducts and 8-oxo-dG increased	(37)
			Negative findings on mutations in other rodent studies	(43,52)
p^{un} allele deletions in mouse embryos	Retinal pigment epithelium	Diesel exhaust particles (transplacental exposure for embryonic days 10.5–15.5 following oral exposure of the pregnant dams)	70 kb deletions spanning exons 6–18 of the p^{un} allele in p^{un}/p^{un} offspring mice. ^{32}P-post-labelling adducts and 8-oxo-dG levels not significantly increased	(53)
p53 gene and *K-ras* gene mutations in rats	Lung tumour tissue	Diesel exhaust; carbon black (inhalation exposure for 24 months)	Infrequent	(54,55)
K-ras gene mutations in rats	Lung adenomas and adenocarcinomas	Diesel exhaust particles (intracheal instillation for 10 weeks, tumours studied after 30 months)	Frequent after intratracheal instillation but not increased after inhalation exposure.	(56)
Promoter methylation[d]				
Human studies				
$p16^{INK4A}$ gene methylation in smoking lung cancer patients	Lung tumour tissue, precursor lesions to lung carcinoma, non-malignant bronchial epithelial cells from brush and sputum samples, and serum DNA	Cigarette smoking	Frequent in NSCLC. Promoter methylation of various other genes also frequently detected	(Reviewed in 100)
$p16^{INK4A}$ gene methylation in cancer-free smokers	Non-malignant bronchial epithelial cells from brush and sputum samples	Cigarette smoking	Present with a lower frequency as compared to the tumour tissue	(103,104,108, 111–113)
Experimental studies				
DAPK gene, and RAR-ß gene methylation in mice	Lung tumour tissue	Cigarette smoke (whole body exposure for 30 months)	Frequent	(116,117)
$p16^{INK4A}$ gene methylation in rats	Lung tumour tissue	Diesel exhaust; carbon black (inhalation exposure for 24 months)	Frequent	(115)

[a] Data available mainly originates from exposure to cigarette smoke (smokers and experimental data).
Abbreviations: NSCLC, non-small cell lung cancer; 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine.

samples from 92 individuals from the same region exposed to coal smoke but with no signs of lung cancer were investigated for presence of mutations (91). *TP53* mutation was found in 15% of these high-risk individuals in non-malignant epithelial cells present in sputum, whereas *KRAS* mutations were less frequent (91). The mutation data associated with indoor emissions from combustion of smoky coal are summarized in Table II.

There are other biomonitoring and molecular data supporting the role of smoky coal emissions in the etiology of these mutations. Measurements of B(*a*)P in the air during cooking, as well as 9-hydroxy-B(*a*)P concentrations in the urine indicated occupational-level exposure, and high levels of various carcinogenic PAH compounds (92,93). The emissions the women were regularly exposed to contained 81% organic matter, of which 43% was PAHs (94). DNA adducts were detected in peripheral white bood cells and placental samples from the exposed women (95), and the presence and quantification of depurinated B(*a*)P-adducted DNA bases in the urine indicated damage due to PAH [B(*a*)P] exposure (96).

In experimental work, organic extracts of indoor air particles from smoky coal emissions were found to induce tumours in mouse skin assay (97). In the *Salmonella* assay, the extract exhibited a mutation spectrum that was consistent with a prominent role for PAHs (94), and GC to TA transversions (78–86%) were the predominant type of mutation. These frequencies are similar to those detected in *Salmonella* after induction by cigarette smoke condensate (78%) and B(*a*)P (77%) (94). The GC to TA transversion frequency in *Salmonella* resembled that observed in *TP53* (76%) and *KRAS* (86%) genes in the lung cancer tissue (88). Furthermore, a recent study suggested that the oxidative pathway of PAH metabolism may also play an important role in the *TP53* mutation spectrum (98).

Promoter hypermethylation and smoking in lung cancer

Aberrant promoter methylation of a number of tumour suppressor genes has frequently been detected in a high percentage (20–100%) of human lung cancers (reviewed in 99,100). Current data reveal promoter hypermethylation as an early event in lung tumorigenesis, and it has been proposed to have clinical importance in lung cancer (99,100). One of the genes frequently inactivated through multiple mechanisms in lung cancer is the *p16* gene ($p16^{INK4a}$/*CDKN2A*), which is involved in inhibition of cell-cycle progression by encoding an inhibitor of cyclin-dependant kinase 4 (CDK4) and 6 (CDK6) (101). In human lung tumours, non-small cell lung cancer in particular, *p16* promoter methylation is common, with ~20–65% of the tumours being positive (99,100).

Both current and former smoking have been associated with aberrant *p16* in lung cancer (99,100,102–109). Methylation of *p16* was increased along with smoking duration, pack-years and smoking during adolescence, and it showed negative correlation with the time since the person quit smoking (105,110). Smokers with lung cancer exhibited aberrant promoter methylation in pre-neoplastic lesions, non-malignant bronchial epithelium cells and serum DNA (99,100,111). Also non-malignant bronchial epithelial cells obtained by bronchial brushes or sputum samples from cancer-free heavy smokers, both current and former ones, have exhibited increased promoter region methylation of several genes, including *p16* (103,104,108,111–113). For lung cancer from non-smokers, varying frequencies of promoter methylation have been reported for several genes (109,112,114). Table II gives an summary of p16 promoter methylation in lung cancer patients and heavy smokers.

The capacity of some airborn particulate carcinogens (including tobacco smoke as a model exposure for PAHs) to induce hypermethylation in the regulatory regions of tumour suppressor genes has been investigated in animal studies (Table II). In rats, particulate carcinogens, such as carbon black and diesel exaust, induced lung tumours of which 46% (carbon black) and 59% (diesel exhaust) showed *p16* methylation (115). Cigarette smoke-induced murine lung tumours have shown high frequencies of gene promoter methylation (116,117). A >50% reduction in lung tumour development was observed in mice after treatment with inhibitors of DNA methylation combined with inhibitors of histone deacetylation (118). From components of air pollution, particulate matter (PM10), as well as nickel and beryllium compounds have been shown to affect histone acetylation status and/or alter DNA methylation patterns (115,119,120). In all, the animal models support involvement of promoter methylation and other epigenetic mechanisms in the modulation of carcinogen-induced lung carcinogenesis (121,122).

Summary and conclusions

On the basis of the recent large cohort studies in the U.S. and in Europe, there are reasonable grounds for concern that air pollution may increase lung cancer risk, especially in combination with other known risk factors, such as active and passive smoking and occupational exposures. Regarding the role of biomarkers, although there are examples of effective contribution of some of them to the understanding of the health effects of air pollution, there are still many aspects that need clarification, in particular reliability of markers. For example, 'bulky' DNA adducts have some degree of batch variation and inter-laboratory variation (123).

DNA damage production reflects primarily carcinogenic exposures, but it is also regulated by inherited and acquired susceptibilities. Indeed, age, gender, BMI, physical exercise, consumption of charcoal-broiled food, consumption of fresh fruits and vegetables and seasonal variations have been reported to influence aromatic DNA adducts. DNA adduct levels have been found to be dependent on polymorphisms in metabolic genes, i.e. CYP1A1 MspI and GSTM1 null genotype (124,125). DNA damage may be repaired, but the ability to remove aromatic DNA adducts may vary from individual to individual.

Numerous experimental studies *in vitro* and *in vivo* have provided unambiguous evidence for genotoxicity of air pollution. In addition to genotoxicity due to the organic extracts of PM (especially various PAH compounds), particulate air pollution induces oxidative damage to DNA (45). This is at least partially assumed to be attributable to the effects of particles *per se* (39,126–128). Both direct effects, i.e. genotoxicity due to inherent physico–chemical properties of particles, and indirect ones, i.e. genotoxicity due to excessive formation of reactive oxygen and nitrogen species by infammatory cell in the course of particle-elicited inflammation, are likely to be involved (39,129,130). However, also soluble chemical substances in air pollution have been shown to induce oxidative damage (24,46), with possible influences from other agents present in polluted air (131,132). The experimental work, combined with the data on frequent oxidative DNA damage in lymphocytes in people exposed to urban air pollution, point to 8-oxo-dG being one of the important promutagenic lesions.

Lung cancer develops through a series of progressive pathological changes occurring in the respiratory epithelium. Molecular alterations, such as loss of heterozygosity, gene mutations and aberrant gene promoter methylation, have emerged as molecular biomarkers of lung carcinogenesis available for studies on groups or individuals at increased risk of cancer, smokers and involuntary smokers in particular (80,87,100,133). Indoor exposure to combustion emissions from smoky coal rich in PAHs is associated with lung cancer that carries *TP53* and *KRAS* mutations, with both genes exhibiting a mutation spectrum typical of PAHs. Gene promoter methylation is common in lung tumours and bronchial epithelium from lung cancer patients who are smokers, and also detectable in variable frequencies in bronchial epithelial cells from cancer-free smokers. Experimentally, particulate carcinogens such as diesel exhaust, carbon black and cigarette smoke have been observed in rodents to induce lung tumours exhibiting frequent aberrant methylation.

Currently, we do not have direct studies on the effects of outdoor air pollution on biomarkers such as tumour mutations or promoter methylation in humans. Although studies on smokers or subjects exposed to indoor emissions rich in PAHs are valuable for understanding common mechanisms of lung carcinogenesis, not necessarily are all these biomarkers optimal for studying effects of outdoor air pollution in humans. In fact, it may be that downstream markers are not sensitive and specific enough for low-dose exposure to carcinogens, such as outdoor air pollution. Therefore, such biomarker studies contribute to carcinogenicity of outdoor air pollution mainly indirectly, via low-dose extrapolation from circumstances of higher exposure.

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