CHAPTER 11. MECHANISTIC CONSIDERATIONS FOR AIR POLLUTION AND LUNG CANCER: GENOTOXICITY AND MOLECULAR BIOMARKER DATA FROM EXPERIMENTAL AND HUMAN STUDIES

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It has long been postulated that lung cancer may result from long-term exposure to ambient air pollution; the actual excess risk has nevertheless been estimated to be considerably less than that associated with tobacco smoking (Higgins, 1976; Pershagen, 1990). In confirmation of the early studies, recent epidemiological investigations have observed an association between outdoor air pollution and lung cancer mortality. It appears that particulate matter (PM), a complex mixture of airborne solid particles and aerosols, is the component causing serious health effects, for example mortality due to cardiovascular diseases and lung cancer (Dockery *et al.*, 1993; Hemminki and Pershagen, 1994; Beeson *et al.*, 1998; Abbey *et al.*, 1999; Cohen, 2000; Pope *et al.*, 2002; Vineis *et al.*, 2004). In particular, long-term exposure to ambient fine particles (aerodynamic diameter < 2.5 μm [$PM_{2.5}$]) has been associated with lung cancer mortality (or incidence) in studies carried out in different parts of the world and among nonsmokers (Dockery *et al.*, 1993; Beeson *et al.*, 1998; McDonnell *et al.*, 2000; Pope *et al.*, 2002, 2004; Laden *et al.*, 2006; Beelen *et al.*, 2008; Katanoda *et al.*, 2011; Turner *et al.*, 2011; Raaschou-Nielsen *et al.*, 2011). One extended follow-up study, the Harvard Six Cities Study from 1974–2009, demonstrated that the association between $PM_{2.5}$ exposure and lung cancer mortality was statistically significant, with a linear concentration–response relationship without a threshold observed down to the $PM_{2.5}$ level of 8 μm/m^3 (Lepeule *et al.*, 2012). In terms of lung cancer deaths, the annual contribution from ambient air pollution to lung cancer mortality has been estimated to be responsible for more than 60 000 deaths worldwide, while more than 700 000 deaths are attributable to cardiac and non-malignant respiratory diseases (Cohen, 2003).

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Sources and constituents of ambient air particulate matter

Airborne particulate pollution is emitted when organic material is burned. There are several major anthropogenic sources of this pollution, such as emissions from traffic (especially diesel-powered vehicles) and other sources such as industrial installations and power stations (see other chapters for more detail). Despite several decades of research, it is not well understood how and why various factors, such as emission site, source, type of emission and exposure concentration, non-particulate fractions and particulate extractable organic matter (e.g. volatile and semivolatile organic compounds), as well as atmospheric transformation products, contribute to the toxicity and biological effects of ambient air pollution (White, 2004; de Kok *et al.*, 2006; Künzli *et al.*, 2006; Claxton and Woodall, 2007; Lewtas, 2007; Steenhof *et al.*, 2011; Benbrahim-Tallaa *et al.*, 2012; also discussed in other chapters).

If one examines the major chemical constituents, then it appears that many complex mixtures from combustion emissions share qualitatively similar profiles; for example, a large number of well-known carcinogens and genotoxicants have been identified in all types of emissions (Claxton *et al.*, 2004: Claxton and Woodall, 2007; Lewtas, 2007; see other chapters). Polycyclic aromatic hydrocarbons (PAHs), in particular mixtures of different PAH compounds, constitute an important class of established genotoxicants and carcinogens (IARC, 1989, 2004; Lewtas and Gallagher, 1990; Boffetta *et al.*, 1997; Claxton *et al.*, 2004; Armstrong *et al.*, 2004; Claxton and Woodall, 2007; Lewtas, 2007; Benbrahim-Tallaa *et al.*, 2012). It is known that genotoxicity and carcinogenicity can also be induced by other chemical components present in ambient air pollution in addition to PAHs (Heinrich *et al.*, 1986; Claxton *et al.*, 2004; Claxton and Woodall, 2007). Differences in the biological effects of these non-PAH components, including their genotoxic and pulmonary inflammatory properties, are to a larger extent dependent on physicochemical characteristics such as particle size and surface area. Moreover, adding to the complexity, the adverse effects of these agents can be mediated via different cellular pathways (Schins, 2002; Donaldson *et al.*, 2002, 2003; Li *et al.*, 2003; Knaapen *et al.*, 2004; Künzli *et al.*, 2006; de Kok *et al.*, 2006; Claxton and Woodall, 2007).

Genetic alterations and epigenetic modifications in human lung cancer

Large proportions of human cancers are sporadic and have mostly an environmental etiology (Lichtenstein *et al.*, 2000; Wogan *et al.*, 2004). A complex, multifactorial disease, human cancer develops through a multistep process with genomic changes representing the driving force. Key oncogenes and tumour suppressor genes (the somatic driver genes) are frequent targets of genetic alterations, in particular mutations (somatic driver mutations) that enable cells to escape growth control and to assume malignant features by disrupting central signalling pathways and networks (Hanahan and Weinberg, 2000; Wood *et al.*, 2007; Hanahan and Weinberg, 2011; Hammerman *et al.*, 2012; Imielinski *et al.*, 2012).

During the past two decades or so, epigenetic modifications – the cancer epigenome – have emerged as another major class of molecular alterations involved in cancer development, with epigenetics taking on a role comparable in significance to that of genetics (Jones and Baylin, 2002; Baylin and Jones, 2011; Berger *et al.*, 2011; Esteller, 2011). Evidence for a close link between the genomic and epigenomic changes has emerged; in other words, genes that directly control the epigenome are also frequent targets of inactivating mutations (You and Jones, 2012).

Recent efforts applying exome and whole-genome sequencing have highlighted the importance of the histone modifier genes as one class of driver mutations in lung cancer (Hammerman *et al.*, 2012; Imielinski *et al.*, 2012).

Along with increasing knowledge of the central molecular features of human cancer, many of the key alterations have become recognized as biomarkers that can be used in translational clinical studies (Baylin and Jones, 2011; Heyn and Esteller, 2012), molecular epidemiology research (Olivier *et al.*, 2010; Herceg and Vaissière, 2011; Schulte *et al.*, 2011), and cancer chemoprevention (Huang *et al.*, 2011). Also, given the fact that it is almost impossible to obtain direct mechanistic information, molecular biomarker data from human lung cancer associated with relevant exposures are of great value when evaluating the evidence for an association between ambient air pollution and the elevated lung cancer risk demonstrated in epidemiological studies.

Aim of the current review

There is wide-ranging and comprehensive literature extending over three decades on the many toxic and other adverse biological effects of exposure to ambient air pollution, including experimental studies conducted both *in vitro* and *in vivo*. There are several extensive and thorough reviews that have compiled and discussed the data and identified the major knowledge gaps that still exist (White, 2004; Claxton *et al.*, 2004; de Kok *et al.*, 2006; Claxton and Woodall, 2007; Benbrahim-Tallaa *et al.*, 2012). Data from human molecular biomarker studies on cancer examining the mechanisms involved in lung carcinogenicity of airborne complex mixtures have focused primarily on *TP53* gene mutations and aberrant DNA methylation of an array of cancer-related genes implicated in human lung cancer. Currently, much of the data on lung cancer originates from smokers, with fewer studies describing lung cancer associated with combustion-related indoor exposure to PAHs. It is more challenging to elucidate the difficult and complex issue of outdoor air pollution and human lung cancer by using the same or similar approaches. This chapter briefly reviews some of those data as a way of providing a mechanistic foundation for the epidemiological findings on the association between air pollution and lung cancer (Vineis and Husgafvel-Pursiainen, 2005; Demetriou *et al.*, 2012). However, no attempt is made to conduct an exhaustive review of the large and continuously growing body of relevant studies; that is beyond the scope of this chapter, and these enormous data sets have been comprehensively and systematically reviewed elsewhere (Claxton *et al.*, 2004; Claxton and Woodall, 2007; Demetriou *et al.*, 2012).

Genotoxicity, mutations, and related biomarkers

Experimental studies on genotoxicity

The properties of outdoor air pollution from anthropogenic, combustion-related sources have been tested for mutagenicity in various systems and experimental settings for many decades. There is now overwhelming evidence that the ambient air contains hundreds of genotoxic compounds. Genotoxicity has been detected in *in vitro* and *in vivo* assays for diesel exhausts, diesel exhaust particles, organic solvent extracts from diesel exhaust PM, urban air particulates, and gasoline exhausts (Ames, 1979; IARC, 1989; Claxton *et al.*, 2004; DeMarini, 2004; de Kok *et al.*, 2006; Claxton and Woodall, 2007; Lewtas, 2007; Benbrahim-Tallaa *et al.*, 2012). However, many of the diverse biological mechanisms underlying the toxic, inflammatory, DNA damaging, and carcinogenic effects still remain unidentified (Krewski *et al.*, 2003; Harrison *et al.*, 2004; Claxton *et al.*, 2004; de Kok *et al.*, 2006; Claxton and Woodall, 2007; Lewtas, 2007).

The mutagenicity of airborne particulates is estimated to be attributable to at least 500 identified components in many different chemical classes (Claxton *et al.*, 2004; Claxton and Woodall, 2007). Furthermore, the size range of airborne particles collected and the chemical reactions occurring in the atmosphere contribute to the complex nature of the genotoxic potential of the ambient air (Claxton *et al.*, 2004; Claxton and Woodall, 2007). For instance, in many studies the quantity of extractable PM, the concentrations of carcinogenic PAHs, as well as the genotoxicity, appear to be higher in winter samples than in summer samples (Binková *et al.*, 1999; Zhao *et al.*, 2002; Farmer *et al.*, 2003; Shi *et al.*, 2003; Castaño-Vinyals *et al.*, 2004; Ramgolam *et al.*, 2009).

In addition to PAH-related damage to DNA, there are abundant data from cell-free systems and experiments using cultured mammalian or human cells revealing that various types of particulates, including diesel exhaust, traffic-related PM/urban dust particles, and wood smoke, can all evoke oxidative stress and subsequent damage to DNA, mainly DNA single-strand breaks or 8-oxo-2′-deoxyguanosine (8-oxo-dG) (Risom *et al.*, 2005, 2007; de Kok *et al.*, 2006; Shi *et al.*, 2006; Danielsen *et al.*, 2011; Benbrahim-Tallaa *et al.*, 2012). Animal experiments have demonstrated that besides diesel exhaust, ambient air can also induce oxidative DNA damage in rodent lung tissue, and some of these effects have been detected at low doses (Nagashima *et al.*, 1995; Ichinose *et al.*, 1997; Tsurudome *et al.*, 1999; Iwai *et al.*, 2000; Sato *et al.*, 2000; Aoki *et al.*, 2001; Risom *et al.*, 2003, 2005, 2007; Dybdahl *et al.*, 2004; Danielsen *et al.*, 2010).

It has been proposed that the oxidative damage related to particulate air pollution is at least partially due to the particles per se – that is to say, the insoluble particle core (Schins, 2002; Donaldson *et al.*, 2003; Karlsson *et al.*, 2004; Knaapen *et al.*, 2004; de Kok *et al.*, 2006; Møller *et al.*, 2010). According to much of the published data, it is both the particulates, in particular the fine dust fraction $PM_{2.5}$, and the soluble chemical substances that are involved in inducing oxidative DNA damage, with possible influences from other components present in the polluted air (Adamson *et al.*, 1999; Bornholdt *et al.*, 2002; Claxton *et al.*, 2004; Risom *et al.*, 2005; Karlsson *et al.*, 2008; de Kok *et al.*, 2005, 2006; Claxton and Woodall, 2007). In general, a crucial role of small PM size fractions (< PM_{10}) has also been recognized for toxicity and genotoxicity of ambient air and traffic-related PM (de Kok *et al.*, 2006; Claxton and Woodall, 2007).

Carcinogenicity and mutations in rodent assays

The carcinogenicity of diesel exhaust has been extensively studied and documented in animal assays over several decades (Heinrich *et al.*, 1986; Mauderly *et al.*, 1987, 1994; IARC, 1989; Mauderly, 1994; Iwai *et al.*, 1997; Pott and Roller, 2005; Lewtas, 2007; Benbrahim-Tallaa *et al.*, 2012); however, only a few studies have applied *in vivo* cancer bioassays to actual ambient air samples (Claxton and Woodall, 2007). If one tries to assess the relative roles of chemical substances versus particulates in carcinogenicity in rats *in vivo*, then it would seem that only 1% of the carcinogenic potency can be explained by organic substances, with only a minimal concentration of adsorbed PAH (Pott and Roller, 2005; Roller, 2009). The strong carcinogenic effect of diesel engine exhaust particles observed in rat inhalation studies is postulated to be due to the small size of the particles (Roller and Pott, 2006; Roller, 2009).

An International Agency for Research on Cancer Monograph Working Group that convened in Lyon in June 2012 concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of whole diesel engine exhaust, diesel engine exhaust particles, and extracts of diesel engine exhaust particles

(Benbrahim-Tallaa *et al.*, 2012). For gasoline exhaust, another significant contributor to urban air pollution, the Working Group concluded that there was *sufficient evidence* in experimental animals for carcinogenicity of condensates of gasoline engine exhaust (Benbrahim-Tallaa *et al.*, 2012).

In vivo transgenic rodent assays have revealed both positive and negative results on the ability of diesel exhaust PM to induce transgene mutations in lung tissue (Sato *et al.*, 2000; Dybdahl *et al.*, 2004; Müller *et al.*, 2004). In the earlier studies, lung tumours from rats exposed by inhalation to diesel exhaust exhibited a low frequency of *Kras* and *p53* gene mutations (Swafford *et al.*, 1995; Belinsky *et al.*, 1997). A high rate of *Kras* mutations in adenomas and adenocarcinomas of the lung was reported after exposure by intratracheal instillation (Iwai *et al.*, 1997).

The human *TP53* knock-in (Hupki) mouse model represents another experimental approach for studying DNA damaging agents (Luo *et al.*, 2001; Olivier *et al.*, 2010; Kucab *et al.*, 2012). This is based on a mouse model with a partial knock-in of the human *TP53* gene and is designed for investigation of *TP53* gene mutations (Luo *et al.*, 2001; Liu *et al.*, 2004). The *in vitro* assay uses immortalized embryonic fibroblasts from the Hupki mouse (HUFs) and has been shown to mimic mutagenesis of the human *TP53* gene (Liu *et al.*, 2004; Olivier *et al.*, 2010). HUFs treated with a mutagen and a suspected human carcinogen present in diesel exhaust and urban ambient air, 3-nitrobenzanthrone (3-NBA), were found to harbour mutations in the human DNA-binding domain of the Hupki *TP53* gene (vom Brocke *et al.*, 2009; Kucab *et al.*, 2010). The most frequently observed mutation was a G:C → T:A transversion, consistent with the presence of persistent 3-NBA-guanosine adducts in the DNA of the exposed cells, and in accordance with earlier studies on 3-NBA-induced mutations. Furthermore, six of these transversions have repeatedly been found in human lung tumours (vom Brocke *et al.*, 2009). In the same manner, an earlier investigation revealed evidence for the induction of human *TP53* gene mutations in HUFs after exposure to benzo[*a*]pyrene (B[*a*]P), another common pollutant in urban ambient air and tobacco smoke (Liu *et al.*, 2005). The B[*a*]P-induced mutations detected in the human *TP53* sequence in HUFs were mainly (41%) G:C → T:A transversions, again in concordance with the *TP53* mutations observed in human lung tumours (Liu *et al.*, 2005). The principal type of mutation (G:C → T:A) found in the human *TP53* sequence in HUFs was also in keeping with the main class of mutations detected in the *cII* gene in the livers of lambda/lacZ transgenic mice (Muta Mouse) exposed intraperitoneally to 3-NBA (Arlt *et al.*, 2004), as well as with the detection of the *gpt* gene mutations in the lungs of another transgenic (*gpt* delta) mouse strain after inhalation of diesel exhaust (Hashimoto *et al.*, 2007).

Germline mutagenicity in animals

In addition to the genotoxicity observed in somatic cells, heritable mutations at repetitive DNA loci have been reported to occur in association with air pollution. A series of studies made use of both experimental and *sentinel* animals to investigate heritable effects after exposure to ambient air at industrial sites and at locations with air pollution from traffic (Somers *et al.*, 2002, 2004; Somers and Cooper, 2009; Somers, 2011).

Laboratory mice were caged outdoors near two integrated steel mills and a major highway in Canada and examined for the presence of expanded simple tandem repeat (ESTR) mutations (Somers *et al.*, 2004). After the mice were housed for 10 weeks at the site, the ESTR mutation rate was increased in comparison with offspring of the unexposed control mice, with the majority of mutations being transmitted through the paternal germline. However, the

mutation rate was reduced by 50%, down to levels measured at a rural reference location, in those animals for whom the air was filtered through a high-efficiency particulate air (HEPA) filter, which removed practically all (> 99%) particles > 0.1 μm in diameter (Somers *et al.*, 2004). The data from laboratory studies with individual chemicals have suggested that the cells sensitive to the induced DNA damage were pre-meiotic germ cells (Vilariño-Güell *et al.*, 2003; Somers *et al.*, 2004). A previous study described a 1.5–2-fold increase in the germline mutation rate at the same repetitive loci in laboratory mice housed at an industrial/urban site compared with rural controls, but the experimental setting did not allow for identification of either the causative agents or the fractions (Somers *et al.*, 2002). Before these studies on laboratory mice housed outdoors in areas with air pollution, a series of experiments on long-lived, non-migratory birds (herring gulls) living near industrial areas consistently observed elevated rates of germline mutations (Yauk and Quinn, 1996; Yauk *et al.*, 2000; Somers and Cooper, 2009).

In a continuation study, mice from an inbred strain (C57BL/CBA) (as opposed to the outbred mice used in the earlier investigation) were exposed *in situ* to ambient air at the same industrial/urban site as in the original study. This study quantified the induced ESTR mutations at three time points, evaluated mutations arising directly in sperm, and characterized DNA lesions (DNA adducts, strand breaks, and global methylation) in the exposed and control (HEPA-filtered air at the same site) animals (Yauk *et al.*, 2008). A 1.6-fold increase in sperm ESTR mutation frequency was detected in mice exposed for 10 weeks, followed by a 6 week break, compared with the control animals, indicating that the mutations had been induced in spermatogonial stem cells. While no bulky adducts were detected in the testes DNA, lung DNA was positive for DNA adducts in the exposed mice compared with control mice caged with HEPA filters. However, strand breaks (at 3 and 10 weeks) were observed in sperm DNA, suggesting that oxidative rather than PAH-related chemical DNA damage had occurred in the mice after the exposure to particles and the associated airborne pollutants. A persistent increase in epigenetic modification (global hypermethylation) in the sperm DNA was also found in mice exposed to ambient air (Yauk *et al.*, 2008). The ESTR mutation induction observed in this study in the sperm of the exposed inbred mice was similar to that detected in the previous study in the offspring of the outbred mice (Somers *et al.*, 2004).

These findings suggest that germline mutagenicity (i.e. induction of mutations that can be passed on to the unexposed next generation) due to air pollution is likely caused by the PM fraction of ambient air; in other words, by mutagens bound to the particles and/or the particles themselves. The central role of particles is supported by the positive findings of strand breaks in the sperm DNA but negative results on PAH-related adducts in the testes DNA in mice caged in a polluted area (Yauk *et al.*, 2008). The mice studies further demonstrate a predominant effect on male germ cells, which is transmissible to the offspring (Somers *et al.*, 2004; Somers and Cooper, 2009; Somers, 2011).

The described series of studies on germ cell mutagenicity of air pollution is supported by investigations demonstrating that tobacco smoke, both mainstream smoke and sidestream smoke, causes germ cell mutations in exposed mice (Yauk *et al.*, 2007; Marchetti *et al.*, 2011). Consequently, it has been postulated that air pollution and tobacco smoke should be classified as germ cell mutagens that may be active at concentrations and through mechanisms also relevant for humans (Demarini, 2012). However, it is of note that multiple mechanisms, not all comprehensively understood, are likely involved in tandem repeat mutagenesis, and, in field experiments, it was not possible to differentiate between true germline mutations that occurred

during gametogenesis and mutations that may have affected early cell divisions in the developing embryos after fertilization (Samet *et al.*, 2004; Somers and Cooper, 2009).

Biomarker studies on genotoxicity in exposed human subjects

Biomarker studies investigating genotoxic effects in various human populations (including studies in children and newborn infants) living in environments with air pollution have been conducted for decades, and many, but not all, have reported positive findings (Perera *et al.*, 1992, 2002; Farmer *et al.*, 1996; Srám *et al.*, 1996; Hemminki and Veidebaum, 1999; Srám and Binková, 2000; Kyrtopoulos *et al.*, 2001; Neri *et al.*, 2006a, 2006b).

DNA damage measured as ^{32}P-postlabelled aromatic adducts has been identified in white cells from peripheral blood of individuals exposed to urban air pollution (Farmer *et al.*, 1996; Peluso *et al.*, 1998; Whyatt *et al.*, 1998; Autrup *et al.*, 1999; Palli *et al.*, 2001; Ruchirawa *et al.*, 2002; Perera *et al.*, 2005; as reviewed in Castaño-Vinyals *et al.*, 2004 and Demetriou *et al.*, 2012). Similar to experimental studies, oxidative DNA damage (8-oxo-dG) and/or DNA single-strand breaks have emerged as an important class of genotoxicity detected in lymphocyte DNA or nasal respiratory epithelium in groups of adults and children with exposure to outdoor air particulates (Calderon-Garciduenas *et al.*, 1996; Valverde *et al.*, 1997; Calderón-Garcidueñas *et al.*, 1999; Loft *et al.*, 1999; Sørensen *et al.*, 2003a, 2003b).

DNA damage was measured by the comet assay in outdoor workers in Mexico City. These workers were found to exhibit significantly higher levels of DNA damage (tail length in comet assay) and a greater percentage of cells with high DNA damage compared with indoor workers (Tovalin *et al.*, 2006). The magnitude of the DNA damage was found to be positively correlated with the exposure of the workers to $PM_{2.5}$ and ozone (Tovalin *et al.*, 2006). In Denmark, nonsmoking bus drivers exposed to urban air pollution (i.e. mainly traffic exhaust fumes) exhibited increased urinary mutagenicity in the *Salmonella* mutagenicity assay compared with mail carriers (Hansen *et al.*, 2004).

Cytogenetic effects (chromosome aberrations, micronuclei, and sister chromatid exchange) have been found in groups of healthy individuals in various geographical locations worldwide. In particular, cytogenetic damage has been observed among traffic policemen in many, but not all, studies. In addition, cytogenetic investigations that have taken into account in the analyses ambient exposure to PAHs, B[*a*]P, or ozone, or effect modification by various susceptibility genotypes have often reported positive findings (Chandrasekaran *et al.*, 1996; Bolognesi *et al.*, 1997a, 1997b; Zhao *et al.*, 1998; Knudsen *et al.*, 1999; Michalska *et al.*, 1999; Burgaz *et al.*, 2002; Carere *et al.*, 2002; Leopardi *et al.*, 2003; Huen *et al.*, 2006; Ishikawa *et al.*, 2006; Sreedevi *et al.*, 2006, 2009; Rossnerova *et al.*, 2009; Rossner *et al.*, 2011). Special attention has been paid to the role of benzene exposure in this context (Hrelia *et al.*, 2004). With regard to mutations, no increase in the frequencies of hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) gene mutations in adults has been found in studies that have included this gene as one of the set of molecular markers being investigated (Farmer *et al.*, 1996; Kyrtopoulos *et al.*, 2001; Perera *et al.*, 2002).

Biomarker studies on exposure of children and newborn infants to ambient air pollution from various urban and other locations, and in different study settings, have reported genotoxicity in a variety of ways, for example DNA adducts (aromatic or PAH–DNA adducts; 8-oxo-dG), protein adducts (albumin or haemoglobin adducts), other DNA damage such as DNA strand breaks, and chromosomal aberrations (Calderón-Garcidueñas *et al.*, 1996, 1997, 1999; Bocskay *et al.*, 2005; Neri *et al.*, 2006a,

2006b; Huen *et al.*, 2006; Orjuela *et al.*, 2010). In particular, studies have investigated pregnant women living in areas with ambient air pollution, often from traffic. In Poland, cord blood samples from newborn infants of mothers living in heavily polluted areas exhibited significantly increased frequencies of aromatic DNA adducts and *HPRT* gene mutations, also seen after adjustment for maternal smoking, suggesting transplacental genotoxicity (Perera *et al.*, 2002). In all, several mother–newborn infant cohorts living in areas with heating- or traffic-related air pollution in Poland, the USA (New York City), and China have consistently reported B[*a*]P-related DNA damage in the newborn infant (leukocytes from umbilical cord blood) in association with maternal exposure to ambient air PAHs (most studies were conducted with nonsmoking mothers); this reflects increased susceptibility of the fetus to DNA damage due to prenatal PAH exposure (Perera *et al.*, 2004, 2005; Jedrychowski *et al.*, 2013; Perera, 2008).

Effects on reproductive health in humans

There are several studies linking exposure to high levels of air pollution with adverse effects on male reproductive health, although with somewhat variable results; mainly damage to sperm DNA, abnormal sperm morphology, and reduced sperm performance have been examined (Selevan *et al.*, 2000; Rubes *et al.*, 2005; Jurewicz *et al.*, 2009; Somers, 2011; Demarini, 2012). There are also reports of a possible influence of genetic polymorphisms on susceptibility to the sperm DNA damage associated with exposure to air pollution (Rubes *et al.*, 2010). In addition, numerous studies have investigated the associations between air pollution and female reproductive health, fecundability, and adverse pregnancy outcomes (Dejmek *et al.*, 1999; Ritz *et al.*, 2002, 2007; Liu *et al.*, 2003; Perera *et al.*, 2003; Srám *et al.*, 2005; Slama *et al.*, 2008; Wilhelm and Ritz, 2005; Wilhelm *et al.*, 2012). One international collaborative study on air pollution and pregnancy outcomes noted the variability in results and study protocols used but reported that 6 (out of 14) studies had found a statistically significant adverse association between an increase in PM_{10} concentration and low birth weight (Parker *et al.*, 2011).

Taken together, the current human biomarker data strongly suggest that genotoxicity (measured as DNA adducts, other DNA damage, cytogenetic effects, urinary mutagenicity) is one of the principal biological mechanisms associated with exposure to ambient air pollution in exposed healthy adults, children, and prenatally exposed newborn infants. There are, however, numerous qualifying issues, including those related to study design, characterization, and concentrations and sources of exposure, as well as issues of individual susceptibility to which attention needs to be paid when carrying out such studies. An accurate assessment of air particulate concentrations is needed to establish exposure–effect relationships. Some biomonitoring studies have included different levels of ambient pollution and carried out personal exposure measurements to overcome this problem (Kyrtopoulos *et al.*, 2001; Sørensen *et al.*, 2003a; Avogbe *et al.*, 2005; Neri *et al.*, 2006a, 2006b). In some studies a correlation has been reported between the extent of the biomarker damage and the level of personal exposure (Sørensen *et al.*, 2003b; Tovalin *et al.*, 2006).

TP53 mutations as a molecular biomarker in human lung cancer

The well-known and most frequently detected genetic alteration in human lung cancer, as in many other cancers, is mutation in the *TP53* gene (Hollstein *et al.*, 1991; Hainaut and Hollstein, 2000; Olivier *et al.*, 2010). Some recent comprehensive efforts with exome and whole-genome sequencing have confirmed the key somatic

driver mutation role of *TP53* in lung cancer (Hammerman *et al.*, 2012; Imielinski *et al.*, 2012).

The spectrum and pattern of *TP53* mutations encountered in human cancers have been widely used as a biomarker in the search for etiological factors involved in the carcinogenic process. As summarized in multiple reviews, there are several unique features that make *TP53* gene mutations a well-suited molecular biomarker for monitoring DNA damage-related human carcinogenesis (Hussain and Harris, 1998; Pfeifer *et al.*, 2002; Olivier *et al.*, 2010; Meek, 2009).

Tobacco smoke (either directly inhaled by the smoker or second-hand smoke) and its various constituents are known to be genotoxic and mutagenic, as has been comprehensively documented (DeMarini, 2004; IARC, 2004; Husgafvel-Pursiainen, 2004). In keeping with this overwhelming evidence, an array of studies has demonstrated an association between mutations of the *TP53* gene and exposure to tobacco smoke in human lung cancer (Hernandez-Boussard and Hainaut, 1998; Hussain and Harris, 1998; Pfeifer *et al.*, 2002; Olivier *et al.*, 2010). The data show that *TP53* mutations occur more frequently in lung cancer among smokers than among never-smokers, and that the frequency of *TP53* mutations is dependent on the daily amount of smoking (Pfeifer *et al.*, 2002; DeMarini, 2004; Husgafvel-Pursiainen, 2004; IARC, 2004). Furthermore, the types and spectrum of mutations in *TP53* are compatible with the presence of PAH-related bulky DNA adducts in the smokers' lung tissue, as well as with the type of DNA damage and mutations known to result from exposure to B[*a*]P and other PAH compounds (Hussain *et al.*, 2001; Hainaut and Pfeifer, 2001; Pfeifer *et al.*, 2002; DeMarini, 2004; Pfeifer and Besaratinia, 2009; Kucab *et al.*, 2010).

Mutations in lung tumours from women exposed to PAHs

TP53 gene and *Kras* gene mutations have been investigated in lung tumours from Chinese (Xuan Wei County) nonsmokers exposed to domestic emissions from unvented firepits or stoves (DeMarini *et al.*, 2001). The indoor combustion emissions from smoky coal contained high levels of PAHs. An exceptionally high mutation frequency (71%), in fact one of the highest frequencies ever reported for lung cancer, was found in the *TP53* gene. The mutations primarily represented the types known to be related to PAH exposure *in vitro* (76% G:C → T:A transversions, with 100% of the guanines involved being on the non-transcribed strand) (DeMarini *et al.*, 2001). Similarly, the mutations in the *Kras* gene, although clearly lower in frequency (29%), were almost entirely G:C → T:A transversions (86%) (DeMarini *et al.*, 2001). A follow-up of these findings in a larger set of lung cancer cases (n = 102), from nonsmoking women exposed to unvented coal smoke in their homes in Xuan Wei County, reported very similar findings (Keohavong *et al.*, 2003). *Kras* mutations were found in 9 women (21.9%), with G:C → T:A transversions accounting for 66.7% of the changes. The frequency and type of *Kras* mutations among the nonsmoking women were comparable to those found in smoking men from Xuan Wei and elsewhere in China (Keohavong *et al.*, 2003). An extension of the study investigated sputum samples from individuals exposed to coal smoke but with no clinical signs of lung cancer. Of the 26 nonsmoking women included in the study, 2 (7.6%) had a *TP53* mutation detected in the non-malignant epithelial cells present in sputum, whereas *Kras* mutations were absent (Keohavong *et al.*, 2005).

There is an impressive amount of evidence proposing that indoor air exposure to PAHs can cause lung mutagenesis and carcinogenesis in nonsmoking women who use smoky coal for cooking and heating in their unvented

homes in Xuan Wei. The lung cancer mortality rates in this county were among the highest for women in China (25.3/100 000; about 8 times the national average for women); almost all (> 99%) of the women were nonsmokers (Mumford *et al.*, 1987). A long-term reduction was observed in the lung cancer incidence in Xuan Wei County after stoves for burning smoky coal were improved by adding chimneys (Lan *et al.*, 2002). In a large retrospective cohort study, domestic use of coal in Xuan Wei County was demonstrated to be linked to highly elevated lung cancer risk, particularly in association with use of smoky coal compared with smokeless coal (hazard ratio for women, 99; 95% confidence interval, 37–266) (Barone-Adesi *et al.*, 2012).

Biomarker studies and chemical analyses have provided further data for the etiological link between lung cancer in women in Xuan Wei and exposure to unvented smoky coal emissions with high levels of various carcinogenic PAH compounds (Mumford *et al.*, 1987). Organic extracts of indoor air particles from smoky coal combustion exhibited tumorigenicity in a mouse skin assay (Mumford *et al.*, 1990). Air measurement of B[*a*]P during cooking and measurements of urinary 9-hydroxy-B[*a*]P concentrations were indicative of high exposure to PAHs. PAH concentrations in indoor air during cooking using smoky coal indicated occupational levels of PAHs (mean concentration for B[*a*]P, 14.6 μg/m^3) (Mumford *et al.*, 1995). The women were regularly exposed to emissions that contained 81% organic matter, of which 43% was PAHs (Granville *et al.*, 2003).

DNA adducts were detected in peripheral blood white cells and placental samples from the exposed women (Mumford *et al.*, 1993), and the presence and quantification of depurinated B[*a*]P-adducted DNA bases in the urine also demonstrated damage due to PAH (B[*a*]P) exposure (Casale *et al.*, 2001). When extracts of smoky coal emissions were tested in various *Salmonella* tester strains, they exhibited a mutagenicity profile that was consistent with that of PAHs (Granville *et al.*, 2003). A prevalence of G:C → T:A transversions (78–86%) was observed that closely resembled those induced by cigarette smoke condensate (78%) and B[*a*]P (77%) (Granville *et al.*, 2003). Again, the frequency of G:C → T:A transversions detected in *Salmonella* was in accordance with the frequencies for *TP53* (76%) and *Kras* (86%) genes observed in lung tumours from the nonsmoking women who had been exposed to coal smoke (DeMarini *et al.*, 2001).

The possible role of genetic variation in xenobiotic-metabolizing genes or in DNA repair genes was investigated in studies of 122 lung cancer patients and 122 individually matched controls from Xuan Wei. The results did point to some protective effects and some associations with elevated lung cancer risk but largely remained suggestive (Lan *et al.*, 2000; Shen *et al.*, 2005a, 2005b). A suggestion of the mechanisms and pathways involved was provided by a study indicating that the oxidative pathway of PAH metabolism is likely to be involved in the *TP53* mutation spectrum and the risk of lung cancer among this population (Lan *et al.*, 2004).

In conclusion, a central role of mutagenesis and carcinogenesis related to exposure to PM rich in PAHs is clear in the etiology of lung cancer among the nonsmoking women in Xuan Wei County, China, who were highly exposed to indoor emissions from combustion of smoky coal. The exceptionally large body of evidence from experimental studies, human biomarker investigations, and epidemiological studies lends support to this conclusion.

Epigenetic changes

Epigenetic modification and environmental exposure

Epigenetics can be defined as the activity of the inherited genome that does not depend on the naked DNA sequence, or as mitotically

and/or meiotically heritable changes in gene function that cannot be explained by changes in the DNA sequence. Epigenetic mechanisms include DNA methylation, histone modification, chromatin remodelling, and non-coding RNAs. These processes have a fundamental function during development and organogenesis, and their abnormal modifications play an important role in cancer (Jones and Baylin, 2002; Baylin and Jones, 2011; Esteller, 2011; Heyn and Esteller, 2012). In addition to aberrant gene promoter hypermethylation, hypomethylation and site-specific demethylation are part of the machinery that may disrupt the normal function of the epigenome (Bhutani *et al.*, 2011; Toraño *et al.*, 2012; You and Jones, 2012). Since the late 1990s, evidence has been accumulating for the role of external exposures in modification and deregulation of the epigenome in human cancer, particularly lung cancer (Belinsky, 2004). It has been proposed that epigenetic mechanisms may function as an interphase between environmental factors and the genome in the cancer process (Herceg and Vaissière, 2011).

In experimental studies, epigenetic alterations, typically altered DNA methylation or histone modification but also other classes of epigenetic modifications (e.g. microRNAs), have been observed *in vitro* in rodent and human cells, as well as *in vivo* in tumour or other tissue from mice and rats after exposure to chemical agents known to be toxic, genotoxic, or carcinogenic. Examples of such exposures include tobacco smoke, carbon black, diesel exhaust, wood smoke, endocrine disrupter chemicals such as bisphenol A and diethylstilbestrol, genotoxic and carcinogenic metals such as chromium, nickel, arsenic, and cadmium, and the tobacco-specific carcinogen NNK (4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone) (Belinsky, 2005; Vuillemenot *et al.*, 2006; Sood *et al.*, 2010; Hou *et al.*, 2012; Feil and Fraga, 2011). In terms of lung carcinogenesis, a crucial role of aberrant DNA methylation has been demonstrated in experimental work, which indicated that inhibition of DNA methylation and histone deacetylation prevent murine lung cancer (Belinsky *et al.*, 2003). Overall, the data from animal experiments strongly support involvement of epigenomic changes in carcinogenesis related to environmental exposure (Fraga *et al.*, 2004; Belinsky, 2005; Hou *et al.*, 2012; Feil and Fraga, 2011).

DNA methylation, lung cancer, and smoking

Hypermethylation of cytosines in CpG-rich islands of gene promoter regions is one of the most studied epigenetic mechanisms (Jones and Baylin, 2002; Jones, 2012). In cancer, hypermethylation of the gene promoter regions is associated with transcriptional inactivation and loss of expression of tumour suppressor and other regulatory genes, thus constituting a mechanism of loss of gene function as an alternative to genetic alterations (Jones and Baylin, 2002; Herman and Baylin, 2003; Jones, 2012). In human cancer, aberrant promoter methylation and other epigenetic modifications occur in a tumour-type and gene-specific manner; in many cancers epigenetic modifications occur early in the tumorigenesis process and may affect a wide range of cellular pathways (Heyn and Esteller, 2012; Baylin and Jones, 2011; Jones, 2012).

Early work indicated that promoter hypermethylation occurs frequently in human lung cancer in the *CDKN2A* (*p16*) gene, as well as in a series of other genes important in the control of cellular growth and proliferation (Merlo *et al.*, 1995; Belinsky *et al.*, 1998; Esteller *et al.*, 1999; Zöchbauer-Müller *et al.*, 2001). Subsequently, a significant association has been detected between tobacco smoking and aberrant promoter hypermethylation in one gene (*CDKN2A*), or multiple cancer-related genes, in lung tumours from cases with non-small cell lung cancer who were current smokers or former smokers. These studies reported significant associations between *p16* methylation and various smoking characteristics

such as duration, pack-years, time since quitting smoking, or smoking in adolescence (Kersting *et al.*, 2000; Palmisano *et al.*, 2000; Kim *et al.*, 2001; Jarmalaite *et al.*, 2003; Toyooka *et al.*, 2003; Belinsky, 2004; Marsit *et al.*, 2005; Vaissière *et al.*, 2009; Heller *et al.*, 2010). A series of studies have further established that tumour suppressor gene hypermethylation can be detected in non-malignant bronchial epithelium from smoking lung cancer patients, as well as in DNA from plasma, serum, or sputum samples from cancer-free smokers; promoter methylation in plasma and sputum increases with lung cancer risk; and promoter hypermethylation in multiple genes in sputum predicts lung cancer (Palmisano *et al.*, 2000; Belinsky, 2004; Belinsky *et al.*, 2005, 2006; Leng *et al.*, 2012).

Recent studies have investigated differential DNA methylation using more epigenome-wide profiling approaches, with variable results. Paired samples of tumour and non-tumour lung tissue were examined for changes in methylation from a total of 146 cases of non-small cell lung cancer (Nelson *et al.*, 2012). The results revealed more than 100 CpG loci with a > 2-fold increase and more than 40 loci with a > 2-fold decrease in methylation from the total studied set of more than 1400 autosomal CpG loci associated with close to 800 cancer-related genes. From these, two genes (*HOXA9*, *SOX1*) showed statistically significant, several-fold increases in methylation, and one gene (*DDR1*) exhibited a significant, several-fold decrease in the level of methylation (Nelson *et al.*, 2012). Another study combined whole-genome DNA methylation analysis with gene expression profiling to investigate lung tumour samples from 50 squamous cell carcinoma cases (Kwon *et al.*, 2012). Thirty hypermethylated and downregulated genes and 22 hypomethylated and upregulated genes were identified. After selection of candidate genes to be targeted, the study reported six genes that were regulated by DNA methylation as based on a demethylation assay; five of these (*CCDC37*, *CYTL1*, *CDOI*, *SLIT2*, *LMO3*) were hypermethylated, whereas one (*SERPINB5*) was hypomethylated (Kwon *et al.*, 2012). Another genome-wide DNA methylation profiling study initially discovered more than 14 000 differentially methylated regions (DMRs) in seven tumour samples of non-small cell lung cancer. After 48 cases of non-small cell lung cancer were studied in more detail, 57 differently methylated regions between paired tumour–non-tumour tissue samples were identified, with some distinct differences between squamous cell carcinoma and adenocarcinoma (Carvalho *et al.*, 2012). The hypomethylated DMRs did not correlate with any particular functional category of genes, while the hypermethylated DMRs were strongly associated with genes encoding transcriptional regulators (Carvalho *et al.*, 2012).

Global methylation has been studied in peripheral blood DNA in smokers, former smokers, and never-smokers. A recent scan of about 27 000 sites in more than 14 000 gene promoter regions in close to 200 individuals identified, with genome-wide significance, that one locus displayed showed lower methylation in smokers (Breitling *et al.*, 2011). The finding was replicated in an independent set of samples analysed with different technologies. The single locus that was hypomethylated in smokers was found to reside in the *F2RL3* (coagulation factor II receptor-like 3) gene region (Breitling *et al.*, 2011).

In contrast to the impressive number of findings among smokers, there are fewer data available on the role of promoter methylation in lung cancer in nonsmokers. The present studies have reported varying frequencies of promoter methylation in nonsmokers (Belinsky *et al.*, 2002; Pulling *et al.*, 2003; Belinsky, 2004; Divine *et al.*, 2005; Sun *et al.*, 2007; Subramanian and Govindan, 2008). An association with exposure to second-hand smoke has been proposed in lung cancer in never-smokers (Scesnaite *et al.*, 2012).

Air pollution and the epigenome

In experimental settings, various types of particulate exposures have been shown to evoke altered DNA methylation, primarily promoter hypermethylation, as briefly described above. With regard to ambient air pollution, a recent study reported that concentrated urban $PM_{2.5}$ increased *p16* promoter methylation in the lungs of mice exposed via inhalation and in primary murine alveolar epithelial cells treated *in vitro* (Soberanes *et al.*, 2012).

In humans, data on epigenetic modifications associated with air pollution have been accumulating during recent years (Christensen and Marsit, 2011; Hou *et al.*, 2012; Jardim, 2011). The association between repetitive element DNA methylation and exposure to particulate emissions from traffic was investigated in blood DNA of more than 700 elderly people living in the Boston, Massachusetts, USA, area. Methylation of the genomic repetitive element LINE-1 was significantly decreased after recent exposure (for 0.5–7 days on average) to carbon black, a PM component of traffic exhaust, and ambient $PM_{2.5}$, with stronger effects observed for the longer time windows (Baccarelli *et al.*, 2009). Among the same study population, prolonged exposure to carbon black and sulfate particles, but not to $PM_{2.5}$, was reported to be associated with hypomethylation of LINE-1 and Alu repeats (Madrigano *et al.*, 2011). In urban traffic officers and gasoline filling station attendants, exposure to low levels of benzene was associated with a significant decrease in global methylation (LINE-1 and Alu repeats) in peripheral blood cell DNA (Bollati *et al.*, 2007).

In the Southern California Children's Health Study, exposure to estimated ambient air PM (PM_{10} and $PM_{2.5}$) was investigated for association with methylation in CpG sites on the three nitric oxide synthase genes (*NOS1*, *NOS2A*, and *NOS3*) in buccal cells from more than 900 children (Breton *et al.*, 2012). $PM_{2.5}$ exposure was found to be associated with different levels of DNA methylation, depending on the NOS gene, the CpG site studied, and the length of exposure. Mostly, but not exclusively, lower methylation levels were observed in association with average 1 year $PM_{2.5}$ exposure (Breton *et al.*, 2012). Another investigation of the same study population reported that an increased 7 day average $PM_{2.5}$ exposure was significantly associated with lower *NOS2* gene (encoding inducible nitric oxide synthase, *iNOS*) promoter methylation, with some interrelated effects of $PM_{2.5}$, *NOS2* promoter haplotypes, and *NOS2* promoter methylation (Salam *et al.*, 2012). In studies of newborn infants (white cells from umbilical cord blood) whose mothers were nonsmokers and lived in New York City, lower global DNA methylation was significantly associated with prenatal PAH exposure but positively linked with the presence of detectable PAH–DNA adducts in cord blood (Herbstman *et al.*, 2012). In the same study population, increased promoter methylation (CpG islands) of the *ACSL3* and *INF-γ* genes in cord blood white cells was associated with maternal PAH exposure (Perera *et al.*, 2009; Tang *et al.*, 2012).

Workers (n = 67) at an industrial estate in Thailand exhibited significantly lower LINE-1, *TP53* gene, and *IL-6* gene methylation, but higher *HIC1* (hypermethylated-in-cancer) gene methylation in blood leukocyte DNA compared with rural residents (n = 45) (Peluso *et al.*, 2012). For all these biomarkers, the residents of the industrial area exhibited methylation levels intermediate between those detected in the workers and in the rural resident controls. Bulky DNA adducts were also assessed and found to be negatively correlated with *TP53* gene methylation (Peluso *et al.*, 2012). In steel plant workers exposed to airborne metal-rich PM, significantly decreased methylation in the *NOS2* gene promoter and the LINE-1 and Alu repeats was observed in peripheral blood cell DNA in association with long-term PM_{10} exposure (Tarantini *et al.*, 2009). In yet another study, workers' exposure to metal-rich PM in

Table 11.1 Summary and examples of positive findings from human biomarker studies investigating combustion-related outdoor or indoor air pollution

Type of damage	Excreta/cell type studied	Main type of particulate exposure	Reference
Bacterial mutagenicity	Urine (adults)	Outdoor air pollution (urban/ traffic exhausts)	Hansen *et al.* (2004)
DNA damage			
Bulky/aromatic or PAH–DNA adducts	White blood cells/lymphocytes from peripheral blood, umbilical cord leukocytes (adults, newborn infants of mothers with exposure)	Outdoor air pollution (urban/ traffic exhausts, industrial site, coal heating)	Farmer *et al.* (1996) Peluso *et al.* (1998) Whyatt *et al.* (1998) Autrup *et al.* (1999) Palli *et al.* (2001) Ruchirawa *et al.* (2002) Perera *et al.* (2005) Demetriou *et al.* (2012)
		Indoor air pollution (emissions from domestic smoky coal combustion)	Mumford *et al.* (1993)
	Placenta	Indoor air pollution (emissions from domestic smoky coal combustion)	Mumford *et al.* (1993)
Oxidative (8-oxo-2′-deoxyguanosine)	White blood cells/lymphocytes from peripheral blood, nasal epithelium (adults, children)	Outdoor air pollution (urban/ traffic exhausts)	Calderøn-Garciduenas *et al.* (1996, 1997, 1999) Loft *et al.* (1999) Sorensen *et al.* (2003a, 2003b) Demetriou *et al.* (2012)
DNA damage/strand breaks/tail length in comet assay	White blood cells from peripheral blood, nasal epithelium (adults, children)	Outdoor air pollution (urban/ traffic exhausts, industrial site)	Valverdere *et al.* (1997) Calderon-Garciduenas *et al.* (1996, 1997, 1999)
DNA fragmentation (%)	Sperm cells	Outdoor air pollution (coal heating, industrial site)	Rubes *et al.* (2005)

Table 11.1 (continued)

Type of damage	Excreta/cell type studied	Main type of particulate exposure	Reference
Cytogenetic effects			
Chromosome aberrations, micronuclei, or sister chromatid exchanges	Lymphocytes from peripheral blood, buccal cells (adults, children)	Outdoor air pollution (urban/ traffic exhausts, industrial site)	Chandrasekaran *et al.* (1996) Zhao *et al.* (1998) Michalska *et al.* (1999) Burgaz *et al.* (2002) Huen *et al.* (2006) Ishikawa *et al.* (2006) Sreedevi *et al.* (2006, 2009) Rossnerova *et al.* (2009)
Gene mutations			
HPRT gene	Lymphocytes from umbilical cord blood (newborn infants of mothers with exposure)	Outdoor air pollution (urban/ traffic exhausts, heating), transplacental exposure	Perera *et al.* (2002)
TP53 gene	Lung tumour tissue (nonsmokers), lung epithelial cells in sputum from nonsmokers with no evidence of cancer	Indoor air pollution (emissions from domestic smoky coal combustion)	DeMarini *et al.* (2001) Keohavong *et al.* (2005)
K-ras (or *NRAS* or *HRAS*) gene	Lung tumour tissue (nonsmokers), lung epithelial cells in sputum from nonsmokers with no evidence of cancer	Indoor air pollution (emissions from domestic smoky coal combustion)	DeMarini *et al.* (2001) Keohavong *et al.* (2003) Keohavong *et al.* (2005)
Differential DNA methylation			
Increased methylation in gene promoter region (*ACSL3* gene, *INF-γ* gene)	Leukocytes from umbilical cord blood (newborn infants of mother with exposure)	Outdoor air pollution (urban/ traffic exhausts)	Perera *et al.* (2009) Tang *et al.* (2012)
Decreased methylation of *NOS1*, *NOS2A*, or *NOS3* gene (various CpG loci or gene promoter)	Buccal cells (children)	Outdoor air pollution (urban/ traffic exhausts, residential communities)	Breton *et al.* (2012) Salam *et al.* (2012)
Hypomethylation of LINE-1 and/ or ALU repeats	White blood cells from peripheral blood (adults)	Outdoor air pollution (urban/ traffic exhausts)	Baccarelli *et al.* (2009) Madrigano *et al.* (2011)
Decreased global DNA methylation	Leukocytes from umbilical cord blood (newborn infants of mothers with exposure)	Outdoor air pollution (urban/ traffic exhausts)	Herbstman *et al.* (2012)

a steel factory was associated with changes in microRNA expression in post-exposure samples of blood leukocytes (Bollati *et al.*, 2010).

Overall summary and conclusions

There is a large body of experimental studies clarifying the mutagenicity, genotoxicity, and male germ cell effects associated with ambient air pollution. Similarly, numerous human biomarker investigations on exposed healthy subjects, both adults and children, have examined DNA damage, other genotoxic and mutagenic effects, and effects on reproductive health effects, as well as epigenetic changes in association with air pollution, as summarized in Table 11.1. These various approaches have provided clear evidence linking air pollution to hazardous biological effects. Data from an abundant number of human lung cancer studies that have investigated mutations or epigenetic alterations in cancer-related genes, in relation to exposure to tobacco smoke or indoor emissions from smoky coal combustion, lend further mechanistic support for this evidence, due to the closely similar nature of these particulate exposures with ambient air pollution. Collectively, the published experimental and human biomarker data, only briefly reviewed in this chapter, clearly associate air pollution with mutagenicity, genotoxicity, and epigenetic modification.

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