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Weight-of-evidence evaluation of associations between particulate matter exposure and biomarkers of lung cancer

H.N. Lynch [a], C.T. Loftus [b], J.M. Cohen [c], L.E. Kerper [a], E.M. Kennedy [a], J.E. Goodman [a, *]

[a] Gradient, 20 University Road, Cambridge, MA 02138, USA
[b] Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, 4225 Roosevelt Way NE, Suite 301 Seattle WA 98105, USA
[c] Gradient, 600 Stewart Street, Suite 1900, Seattle, WA 98101, USA

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ABSTRACT

Research suggests that exposure to ambient particulate matter (PM) may be associated with lung cancer; however, no mode of action (MoA) for this has been established. We applied a weight-of-evidence (WoE) approach to evaluate recent evidence from four realms of research (controlled human exposure, epidemiology, animal, and *in vitro*) to determine whether the overall evidence supports one or more MoAs by which PM could cause lung cancer. We evaluated three general MoAs: DNA damage and repair; other genotoxic effects, including mutagenicity and clastogenicity; and gene expression, protein expression, and DNA methylation. After assessing individual study quality, we evaluated the strength of the evidence within as well as across disciplines using a modified set of Bradford Hill considerations. We conclude that the overall WoE indicates it is plausible that PM of various size fractions may cause direct DNA damage, but the evidence is insufficient regarding the alternative MoAs we evaluated. More research is needed to determine whether DNA damage can lead to downstream events and, ultimately, lung cancer.

1. Introduction

[1]Particulate matter (PM) is a generic term for a large class of chemically and physically diverse substances that exist as liquid droplets or solid particles in various size fractions. There are both anthropogenic and natural sources of PM. Anthropogenic sources include stationary sources, such as fossil-fuel burning power plants and factories, and mobile sources (predominantly vehicles), while natural sources include forest fires and wind-blown soil. PM is also formed in the atmosphere following chemical reactions of gaseous pollutants such as sulfur oxides, nitrogen oxides, and volatile organic compounds (US EPA, 2009). The Clean Air Act mandates that the United States Environmental Protection Agency (US EPA) set health-based National Ambient Air Quality Standards (NAAQS)

* Corresponding author.
E-mail address: jgoodman@gradientcorp.com (J.E. Goodman).

[1] 8-OHdG = 8-Oxo-2'-deoxyguanosine; Alu = *Arthrobacter luteus* Restriction Endonuclease; ANOVA = Analysis of Variance; *Anxa5* = Annexin A5; ARRIVE = Animal Research: Reporting of *In Vivo* Experiments; BALF = Bronchoalveolar Lavage Fluid; BC, black carbon; BMI = Body Mass Index; CI = Confidence Interval; CAP = Concentrated Ambient Particle; CV = Coefficient of Variation; DEP = Diesel Exhaust Particle; EC = Elemental Carbon; εdA = Etheno-DNA Adducts; EGR-1 = Early Growth Response 1; ESTR = Expanded Simple Tandem Repeat; FA = Filtered Air; FPG = Formamidopyrimidine DNA Glycosylase; GD = Gestation Day; GEE = Generalized Estimating Equation; GLM = Generalized Linear Model; HBE = Human Bronchial Epithelial; HEPA = High-Efficiency Particulate Arresting; HSP = Heat Shock Protein; iNOS = Inducible Nitric Oxide Synthase; ISA = Integrated Science Assessment; LINE-1 = Long Interspersed Nuclear Element 1; LMM = Linear Mixed Model; *miR* = MicroRNA; MoA = Mode of Action; NAAQS = National Ambient Air Quality Standards; $NC_{0.1\text{-}0.5}$ = Number Concentration in the Size Range 0.1–0.5 μm; $NC_{0.01\text{-}0.1}$ = Number Concentration in the Size Range 0.01–0.1 μm; NF-κB-*p50* = Nuclear Factor-Kappa B, *p50*; NIST SRM = National Institute of Standards and Technology Standard Reference Material; *NQO1* = NAD(P)H Dehydrogenase, Quinone 1; OC = Organic Carbon; OE = Organic Extract; OECD = Organization for Economic Co-operation and Development; *OGG1* = 8-oxoguanine DNA Glycosylase; OR, odds ratio; PAH = Polycyclic Aromatic Hydrocarbon; PAR = Poly(ADP-ribose); PM = Particulate Matter; PM_1 = Particulate Matter <1 μm in Diameter; $PM_{2.5}$ = Particulate Matter ≤2.5 μm in Diameter; PM_4 = Particulate Matter <4 μm in Diameter; PM_{10} = Particulate Matter ≤10 μm in Diameter; QA = Quality Assurance; QC = Quality Control; ROS = Reactive Oxygen Species; SCE = Sister Chromatid Exchange; *TLR-2* = Toll-Like Receptor 2; TPN = Total Particle Number; UFP = Ultrafine Particle ≤0.1 μm in Diameter; US EPA = United States Environmental Protection Agency; WoE = Weight of Evidence; WHO = World Health Organization.

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for six criteria air pollutants, including PM. At present, there are standards for $PM_{2.5}$ ($\leq$2.5 μm in diameter) and PM_{10} ($\leq$10 μm in diameter). Although no standards exist for other size fractions, US EPA has evaluated causal relationships between $PM_{10\text{-}2.5}$ (2.5–10 μm in diameter) and ultrafine particles (UFPs, $\leq$0.1 μm in diameter) and health effects (US EPA, 2009).

In the 2009 Integrated Science Assessment (ISA) for Particulate Matter, US EPA determined that the evidence was inadequate to determine whether there was a causal association between $PM_{10\text{-}2.5}$ and UFPs and cancer. US EPA also concluded that the evidence was suggestive of a causal relationship between $PM_{2.5}$ exposures and cancer and indicated that the strongest evidence for this association came from epidemiology studies of lung cancer mortality. US EPA noted that studies showed that gasoline and diesel exhaust are mutagenic and polycyclic aromatic hydrocarbons (PAHs) are genotoxic, providing biological plausibility for the epidemiology evidence. It stated, however, that it was unclear how other changes seen in mechanistic studies, such as alterations in gene methylation, could influence the initiation and promotion of cancer.

At the time of the release of the ISA, mechanistic literature on associations between PM and cancer was somewhat limited, particularly for environmentally relevant exposure levels. US EPA summarized early mechanisms whereby PM could cause a variety of health effects, focusing on the generation of reactive oxygen species (ROS) and early sequelae (*e.g.*, activation of cell signalling pathways and pulmonary inflammation). As discussed in Section 2.2.3, these mechanisms are not specific to cancer. In their discussion of the potential for PM-induced cancer, US EPA briefly discussed mutagenicity in animal and *in vitro* assays (*e.g.*, in *Salmonella typhurium*) and markers of susceptibility in humans and animals (*e.g.*, micronuclei). As discussed further in Section 4, US EPA reviewed approximately seven human studies of DNA damage and related outcomes, reporting inconsistent results after exposure to $PM_{2.5}$, PM_{10}, and UFP. Further, several of the studies US EPA reviewed were not designed to assess the quantitative relationship between increases in PM, specifically, and DNA damage. US EPA (2009) also summarized several *in vitro* studies showing associations between PM and "PM-associated constituents" and micronuclei, DNA adducts, sister chromatid exchanges (SCEs), DNA stand breaks, frameshifts, and inhibition of gap-junction intracellular communication, though many of these studies are not discussed in detail. US EPA concluded that "studies of exposure/susceptibility markers have reported inconsistent outcomes, with some markers being associated with PM and others not" (US EPA, 2009).

Finally, US EPA also reported that $PM_{2.5}$ "potentially affects some DNA methylation content," but noted that it could not determine how the reported genomic alterations could influence initiation and promotion of cancer. Overall, although US EPA indicated that the available studies provided some evidence to support the biological plausibility of PM-lung cancer relationships, this evidence was mainly from *in vitro* studies.

Since the 2009 ISA, the body of mechanistic studies has expanded greatly, enabling more complete assessment of the MoAs underlying the potential associations between particulate matter (UFPs, $PM_{2.5}$, and PM_{10}) and lung cancer. Therefore, in this review we examine whether studies of cancer biomarkers support a biologically plausible MoA by which PM (and specifically, $PM_{2.5}$, PM_{10}, and UFPs) could cause lung cancer.

1.1. Cancer

Carcinogenesis is a complex, multi-step process whereby normal cells become abnormal, malignant derivatives. Cancer cells are characterized by six essential alterations in cell physiology that allow this malignant growth: self-sufficiency in growth signals (*i.e.*, they generate their own growth signals), insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), unchecked replication/proliferation, sustained angiogenesis (development of new blood vessels), and tissue invasion and ability to spread (metastasis) (Hanahan and Weinberg, 2000, 2011).

Each cell in the human body develops tens of thousands of DNA lesions per day. These are formed during the course of normal processes such as oxidative respiration and are also produced by macrophages and neutrophils. They can also occur after exposure to exogenous chemical substances. To respond to these insults, the body has numerous, largely distinct DNA repair mechanisms (*e.g.*, mismatch repair and base excision repair). Chronic DNA damage that cannot be repaired triggers cell death by apoptosis or cellular senescence (permanent withdrawal from the cell cycle) (Jackson and Bartek, 2009). DNA repair mechanisms are activated in early neoplastic lesions and likely protect against malignancy, but alterations in DNA damage response pathways may breach this barrier and allow malignant progression (Jackson and Bartek, 2009). The MoA of many carcinogens is generating direct DNA damage and causing genetic mutations. Others may operate *via* epigenetic MoAs, in which they alter DNA methylation and/or the expression of genes (including oncogenes and tumor suppressors) that control cellular activities, but do not alter the underlying DNA. For example, activation of oncogenes or inactivation of certain tumor suppressor genes can cause aberrant cell proliferation, which then elicits DNA replication stress and ongoing DNA damage (Jackson and Bartek, 2009).

1.2. Biomarkers of cancer

Biomarkers are measures of biological events that take place in the continuum between exposure to a substance and a disease of interest. Most often, biomarkers are measured in easily collected and analyzed biological samples, including urine and blood. Some biomarkers indicate exposure (*e.g.*, detection of a chemical metabolite), and others represent various stages in a given disease process, including early or late biological changes (Boffetta, 2010). A specific biomarker's usefulness in predicting cancer is related to where on the specific pathway to disease it occurs and the sensitivity and specificity by which it can be measured, including whether it is associated with etiologic changes specific to the cancer process relative to other disease states. In many cases, biomarkers are more reliable if they occur further along in the process of cancer development (Au, 2007). In addition, regardless of the timing, biomarkers of highly specific, relatively difficult to repair (or irreparable) effects typically predict future cancer risk with more certainty; examples of these better-validated biomarkers include chromosomal aberrations (Au, 2007). However, substantial inter-individual variability exists in human susceptibility to any given exposure and, therefore, to the development of biomarkers of effect – factors that affect susceptibility include genetics, lifestyle choices (smoking, diet, *etc.*), age, and health history (*e.g.*, chronic infection) (Au, 2007).

The use of biomarkers in cancer epidemiology and toxicology has increased, particularly as knowledge of the mechanisms of carcinogenesis expands, and new assays and models of molecular biology, and of genetic and epigenetic events, emerge. Despite these advances, cancer etiology is complex, and the current understanding of cancer epidemiology remains limited; few environmental pollutants have been definitively linked with increased cancer risk (Boffetta, 2010). The next three sections provide a short discussion of common cancer biomarkers that we included in our analysis; a summary of the genes and protein biomarkers we reviewed and their roles is presented in Table 1.