UNITED STATES COURT OF APPEALS
FOR THE DISTRICT OF COLUMBIA CIRCUIT

No. 15-1363 September Term, 2015

EPA-80FR64662

State of West Virginia, et al.,

Petitioners

v.

Environmental Protection Agency et al.,

Respondents

MOTION TO INTERVENE OF WEST VIRGINIA NON-GOVERNMENTAL ORGANIZATIONS

The West Virginia Highlands Conservancy, the Ohio Valley Environmental Coalition, Coal River Mountain Watch, the Kanawha Forest Coalition, Mon Valley Clean Air Coalition and Keepers of the Mountains Foundation -- all West Virginia-based non-governmental organizations (WV NGOs) -- respectfully request that this Court enter an order permitting them to intervene in this proceeding. In support of this motion, WV NGOs state as follows:

1. The moving WV NGO's support the provisiions of the CPP and will be directly affected by the outcome of this litigation.

West Virginia relies upon coal for more than 96% of the electricity generated in the state – a greater dependence than any other state in the nation -- and, as a direct consequence of that disporportionate dependence, leads the nation in deaths per 100,000 citizen from particulate matter emitted from coal-fired electric generation plants, which plants.

2. The challenged Clean Power Plan (CPP) adopted by the United States Environmental Protection Agency (EPA), if upheld in this proceeding and implemented by the State of West Virginia, would lead to a significant reduction of the use of coal by electric generation units, with a resulting direct and immediate positive impact on the health of West Virginia citizens. Alternatively, if this Court were to invalidate or delay the implementation of the CPP, the health of these intervenors and numerous other citizens of West Virginia would be severely and adversely affected.

3. On August 24, 2015, all but one of the moving WV NGO's seeking to intervene in this proceeding, filed a memorandum and supporting evidentiary materials with the EPA in opposition to the August 5, 2015 request for administrative stay, submitted in the name of the "State of West Virginia" and fifteen other states, seeking to

delay until the conclusion of judicial review the CPP, then adopted as a final rule but not yet published in the Federal Register.

4. The named NGOs, with a collective membership in excess of 1,000 West Virginians, move to intervene because any delay in the implementation of the CPP pertaining to carbon pollution emission guidelines for electric utility generating units will expose them and their fellow citizens to unwarranted risk of disability and/or death.

5. The intervening WV NGO's include the following:

a. The West Virginia Highlands Conservancy (www.wvhighlands.org) has since 1965 provided its 700 members and the citizens of West Virginia advocates for protection of public resources, resists the exploitation and destructive of Appalachian mountains by mountaintop removal to fuel coal-fired electric generation plants, and supports the environmental justice and health objectives of the CPP.

b. The Ohio Valley Environmental Coalition (OVEC) (www.ohvec.org), is a grassroots organization with approximately 400 members headquartered in Huntington, WV. OVEC's members organize West Virginia citizens to resist destruction of West Virginia's mountains in order to provide raw materials used to

operate coal-fired electric generation plants. OVEC advocates for the preservation of West Virginia's environment and mountain communities through education, grassroots organizing and supports the environmental justice and health objectives of the CPP.

c. Coal River Mountain Watch, Inc. (www.crmw.net), a non-profit corporation based in Naoma, WV, has worked since 1990 to end the destruction of West Virginia communities and environment by advocating the abolition of the coal mining practice of mountaintop removal (MTR), a coal mining practice which supplies fuel for coal-fired electric generation plants. Coal River Mountain Watch advocates before public officials and agencies for sustainable forms of economic development and supports the environmental justice and health objectives of the CPP.

d. Kanawha Forest Coalition (https://www.facebook.com/kanawhaforestcoalition/) is an unincorporated association of approximately 50 citizens of West Virginia who organized in 2013 to oppose the surface coal mining in very close proximity to densley populated residential neighborhoods, and immediately adjacent to a state forest, and supports the environmental justice and health objectives of the CPP.

e. Mon Valley Clean Air Coalition (http://www.monvalleycleanair.org/) is a West Virginia non-profit corporation, organized in 2008, to promote clear air in northern West Virginia and southwestern Pennsylvania, with a focus on the Monongahela River watershed. Mon Valley Clean Air Coalition supports the environmental justice and health objectives of the CPP.

f. Keepers of the Mountains Foundation (www.mountainkeeper.org) a non-profit corporation, organized in West Virginia, with its headquarters in Charleston, WV, has since its organization in 2004, participated in legal proceedings and community organization for the purpose of ending the destruction of Appalachian mountains to supply fuel to coal-fired electric generation plants. KOTM supports the environmental justice and health objectives of the CPP.

6. Further, this motion to intervene is the only pleading filed in this proceeding on behalf of individual West Virginia citizens with a personal interest in the outcome of the litigation. Under the terms of the CPP as finally adopted by the EPA, the governor of a state is charged with developing and filing with EPA a State Implementation Plan. Under the terms of West Virginia law, the Governor is required

to prepare a proposed SIP, with the assistance of the W. Va. Department of Environmental Protection (DEP), which is then submitted to the W. Va. legislature for review, prior to submission to the EPA. And under West Virginia law, , codified at W. Va. Code § 5-3-1, the Governor or the DEP may request, in writing, legal representation by the state Attorney General in order to commence litigation, such as the current Petition for Review of the CPP filed in this Court on October 23, 2015.

7. However, documents released by by the Governor (**Exhibit A)** and the DEP (**Exhibit B)**, and confirmed by additional documents released the state Attorney General (**Exhibit C**), establish unambiguously that neither the Governor nor the WV Department of Environmental Protection requested the Attorney General -- in writing or otherwise -- to represent them or the "State of West Virginia" in this proceeding. To the contrary, the Governor of the State of West Virginia – charged by the CPP with the preparation and filing of a SIP to provide for compliance with the CPP -- has publicly announced his intention to prepare a SIP to comply with the CPP (**Exhibit D)**, and the DEP has solicited comments from the public through December 31, 2105 to assist it in preparation of a SIP. (**Exhibit E)**,

8. The Petition for Review filed by the W. Va. Attorney General, purporting to speak for the "State of West Virginia," asserts gratuitously that the final CPP rule will require the state to "reduces statewide carbon dioxide emission from coal-fired power plants by a staggering average of 32% from 2005 levels, in just 15 years." In fact, changes already caused by the impact of lower prices for natural gas, have already accounted for a 20% decline in coal-fired electric generation – from 44.6% of the market to 36% – in the first quarter of 2011 alone, long before the final rule was promulgated.

9. As noted, the Governor, with the assistance of the DEP, and the review of the W. Va. legislature, will make the ultimate decision for the State of West Virginia regarding the CPP. The State Governor, charged with implementation of the CPP, has publicly repudiated the Petitioning WV Attorney General's claim that the State pf West Virginia is Incapable of complying with the CPP. Moreover, West Virginia law accords no role to the Attorney General in the decision making process for the State of West Virginia as it pertains to the CPP.

10. The WV Attorney General's Petition for Review in this matter is nothing more than the expression of the ideological views of

a political figure with no official role in the state's CPP decision making process, and no personal stake in the outcome of this litigation. No general statutory authority of the Attorney General can satisfy the agency, i.e., attorney-client relationship, required in *Hollingsworth v. Perry*, 133 S.Ct. 2652, 2667 (2013), for a finding of standing and, as a consequence, to provide this Court jurisdiction over a "case and controversy" for Article III purposes. In the absence of an engagement by a State official charged with at least minimal decision making authority regarding the CPP, the Attorney General's Petition for Review in Case No 15-1363 constitutes no more than a statement of the individual signatory's personal political ideology. The Petition for Review filed by the Attorney General in the name of the "State of West Virginia" be dismissed by this Court, *sua sponte.* See *Hollingsworth v. Perry*, 133 S.Ct. 2652, 2667 (2013).

11. By contrast, the moving WV NGO's and their families have a direct personal interest which will be immediately impacted by the outcome of this litigation, whether the CPP is upheld or struck down. The adverse health impacts of coal use, observed anecdotally for more than a century, have been the subject of modern epidemiological studies, and the empirical findings are unambiguous.

In literally dozens of recent peer-reviewed studies, diligent medical researchers have documented the fact that particulate matter -- whether emitted directly from electric utility plants, or indirectly from the mountaintop removal mining projects from which W. Va. utilities obtain 96% of their fuel supply -- results in statistically significant increases of birth defects, decreased birth weights, diminished educational attainment, increased cancer, pulmonary and cardiac disease, and very substantially decreased life expectancy.

12. Specifically, in the article entitled "*Atmospheric particulate matter size distribution and concentration in West Virginia coal mining and non-mining areas*," attached as **Exhibit F** and published online on February 19, 2014 in the Journal of Exposure Science and Environmental Epidemiology (2014) 24, 405-411, the authors – all medical researchers at the West Virginia University School of Public Health – conclude that:

a. exposure to increased particulate matter is associated with excess respiratory and cardiovascular hospital admissions, morbidity, and mortality;

b. coal mining, including mountaintop removal coal mining, was prominent around the higher disease rate area and no mining occurred in the lower disease rate area;

c. residents of coal mining areas have significantly higher mortality from chronic heart, respiratory, and kidney

diseases, and elevated morbidity from chronic cardiopulmonary, cardiovascular, and kidney diseases;

d. age-adjusted total mortality rated in mountain top removal coal mining areas is significantly greater compared with non-mining areas in central Appalachain states.

e. residents of mountain top removal mining areas have an increased prevalence of congenital anomaly births compared with residents of other Appalachian areas;

f. age-adjusted chronic cardiovascular rates are greater in mountain top removal coal mining counties compared to counties without such coal mining.

13. In connection with these observations, the peer-reviewed studies note that size variation in the particulate matter studied indicated sources other than mountaintop removal mining, and that secondary particulate matter in the studied coal mining communities, could be accounted for by the presence of coal-fired electric plants in Cincinnati, Ohio; Columbus, Ohio; Lexington, Kentucky and the Ohio River Valley areas located northwest and south of the sampling areas.

14. Particularly relevant for the current proceeding, the authors further note that "Coal-fired power plant emissions cause health problems among population living near and at a distance from plants," citing Levy JI, Spengler JD. "*Modeling the benefits of power*

plant emissions controls in Massachusetts," J Air Waste Manage Assoc 2002; 52: 5–18, and MacIntosh DL, Levy JI, Spengler JD. Testimony before the Wisconsin Public Service Commission: Matter of a Pollution Control Construction Permit, Case No. IH-04-03 2003.

15. The fact that repeated independent peer-reviewed studies consistently observe statistically significant associations between coal-derived particulate matter and adverse health affects, is not the sole basis for the conclusion that delay of the final rule will place American citizens' health at risk.

16. Medical researchers at the West Virginia University School of Public Health have also isolated the biological mechanisms which tie coal-derived particulate matter to specific diseases. In "*Appalachian Mountaintop Mining Particulate Matter Induces Neoplastic Transformation of Human Bronchial Epitheliala Cells and Promotes Tumor Formation*," attached as **Exhibit G**, and published in the Environmental Science & Technology Journal on October 14, 2014, the researchers' found that:

> [C]hronic exposure (3 months) to non-cytotoxic, physiological relevant concentration (1μg/mL) of PMMTM, but not control particle PMCON, induced neoplastic transformation, accelerated cell proliferation and enhanced cell migration of the exposed lung cells. Xenograft transplantation of the PMMTM-exposed cells in

> mice caused no apparent tumor formation, but promoted tumor growth of human lung carcinoma H460 cells, suggesting the tumor promoting effect of PMMTM. Chronic exposure to the main inorganic chemical constituent of PMMTM, molybdenum but not silica, similarly induced cell transformation and tumor promotion, suggesting the contribution of molybdenum, at least in part, in the PMMTM effects. These results provide new evidence for the carcinogenic potential of PMMTM and support further risk assessment and implementation of exposure control for PMMTM.

Exhibit G.

17. The implicatons of these findings are straight forward. Failure to implement the CPP, even minimal delays of a few months, will expose the moving WV NGO's, their families and many thousands of other citizens of the United States, to high risk carcogenic toxins. Any effort to interrupt the earliest possible implementation of the CPP is unreasonable as a matter of law, scientfically unsupportable and morally indefensible. The intervention of the moving WV NGO's is necessary to accurately present the interest of West Virginia citizens, and will ably demonstrate for this Court the foundation for the environmental justice ground upon which the EPA explicitly based its adoption of the CPP.

WHEREFORE, the West Virginia NGOs respectfully that their motion to intervene by granted.

Respectfully submitted,

WEST VIRGINIA HIGHLANDS CONSERVANCY,
OHIO VALLEY ENVIRONMENTAL COALITIION,
COAL RIVER MOUNTAIN WATCH, INC.,
KANAWHA FOREST COALITION,
MON VALLEY CLEAN AIR COALITION and
KEEPER OF THE MOUNTAINS FOUNDATION

By Counsel

William V. DePaulo, Esq.
122 N Court Street, Suite 300
Lewisburg, WV 24901
Tel: 304-342-5588
Fax: 866-850-1501
william.depaulo@gmail.com

CORPORATE DISCLOSURE STATEMENT

Pursuant to Fed. R. App. P. 26.1 and L.R. 26.1, each of the the West Virginia Non-Governmental Organizations (NGOs) moving for intervention, hereby individually certifies that there is no parent or publicly-held corporation or other entity that has a 10% or greater ownership of any one of any individual NGOs. None of the moving NGO's have members with an ownership interest, and no member of an unincorporated entity has issued shares or debt securities to the public. The interest of each NGO in this litigation is to insure that environmental justice principles of the CPP are upheld, and the health and other environmental benefits of the CPP are secured, by the outcome of this litigation.

November 23, 2015

William V. DePaulo

CERTIFICATE OF SERVICE

I hereby certify that a copy of the foregoing Motion to Intervene and Corporate Disclosure Statement was, this 23rd day of November, 2015, filed electronically with the Clerk of the United States Court of Appeals for the District of Columbia Circuit via the CM/ECF filing system and thereby served on all parties in this case and the consolidated cases.

William V. DePaulo

STATE OF WEST VIRGINIA
OFFICE OF THE GOVERNOR
1900 KANAWHA BOULEVARD, EAST
CHARLESTON, WV 25305
(304) 558-2000

EARL RAY TOMBLIN
GOVERNOR

October 29, 2015

Mr. William V. DePaulo, Esq.
122 N. Court Street, Suite 300
Lewisburg, West Virginia 24901

Dear Mr. DePaulo:

The Office of the Governor received your email of October 27, 2015, in which you requested, pursuant to the Freedom of Information Act, W. Va. Code § 29B-1-1, *et seq.*, the following documents:

> [W]ritten requests pursuant to W. Va. Code 5-3-1, by the Governor of the State of West Virginia, addressed to Patrick Morrissey [sic], the Attorney General of the State of West Virginia, or to Elbert Lin, the Solicitor General of the State of West Virginia, requesting that the Office of Attorney General represent the Governor or any other state officer, board or commission, in Case No. 15-1363, styled "State of West Virginia, et al." filed with the United States Circuit Court of Appeals for the District of Columbia Circuit, filed October 23, 2015, and consisting of a Petition for Review of the so-called "Clean Power Plan" adopted by the United States Environmental Protection Agency.

Please be advised that we have not located any records that are responsive to your request. Accordingly, your request is denied and our responsibilities in connection therewith are at an end. You are further advised you have the opportunity to institute proceedings for injunctive or declaratory relief in the Circuit Court of Kanawha County.

Sincerely,

Peter G. Markham

Peter G. Markham
General Counsel

west virginia department of environmental protection

Executive Office
601 57th Street, Southeast
Charleston, West Virginia 25304
Phone: (304) 926-0440
Fax: (304) 926-0446

Earl Ray Tomblin, Governor
Randy C. Huffman, Cabinet Secretary
www.dep.wv.gov

October 9, 2015

William DePaulo, Esquire
122 N. Court Street, Suite 300
Lewisburg, WV 24901
Via Electronic Mail Only: william.depaulo@gmail.com

Re: FOIA Request No. 2015-10-036

Dear Mr. DePaulo:

In response to your FOIA request dated October 7, 2015 requesting

> Copies of all public records pertaining to, or constituting, a writing from the Department of Environmental Protection (DEP) addressed to Patrick Morrissey, Attorney General of West Virginia, or Elbert Lin, Solicitor General of West Virginia, requesting that the Attorney General provide legal representation of the DEP in connection with litigation relating to the so-called "Clean Power Plan," a ruled adopted on August 3, 2015 by the United States Environmental Protection Agency (EPA) entitled "CARBON POLLUTION EMISSION GUIDELINES FOR EXISTING STATIONARY SOURCES," in EPA docket no. EPA-HQ-OAR-201 3-0602; RIN 2060-AR33.

please be advised that there are no such records.

If you have any questions or concerns, or if you wish to discuss this matter in any particular, please do not hesitate to contact me.

Very truly yours,

Kristin A. Boggs
General Counsel, Acting Chief of the
Office of Legal Services

State of West Virginia
Office of the Attorney General

Patrick Morrisey
Attorney General

(304) 558-2021
Fax (304) 558-0140

October 28, 2015

William V. DePaulo, Esquire
122 N Court Street
Suite 300
Lewisburg, WV 24901
Via email: william.depaulo@gmail.com

Re: FOIA Request Dated October 7, 2015
AG FOIA Docket # 15-WD-001

Dear Mr. DePaulo:

In compliance with the West Virginia Freedom of Information Act, W.Va. Code § 29B-1-1 *et seq*., this office searched its records following receipt of the above-reference request. The purpose of this letter is to respond to your request on behalf of the West Virginia Office of Attorney General. Each request is set forth below together with this Office's response:

1. Written requests pursuant to W. Va. Code 5-3-1, by the Governor of the State of West Virginia, the Department of Environmental Protection (DEP) or any other state officer, board or commission, or the head of any state educational, correctional, penal or eleemosynary institution, addressed to Patrick Morrisey, the Attorney General of the State of West Virginia, or to Elbert Lin, the Solicitor General of the State of West Virginia, requesting that the Office of the Attorney General represent the Governor, the DEP or any other state officer board or commission, in connection with litigation relating to the so-called "Clean Power Plan," a rule adopted on August 3, 2015 by the United States Environmental Protection (EPA) entitled "CARBON POLLUTION EMISSION GUIDELINES FOR EXISTING STATIONARY SOURCES, in EPA docket no. EPA-HQ-OAR-201 3-0602; RIN 2060-AR33.

Response: This Office maintains no public documents responsive to this request.

(2) Any other authority, statutory or otherwise, pursuant to which the Office of the Attorney General has entered, or will enter, appearances as counsel for the "State of West Virginia" in litigation before EPA or in the federal court system, relating to the Clean Power Plan, including but not limited to:

(a) Case No. 14-1146 styled State of West Virginia, et al., v Environmental Protection Agency, before the United States Court of Appeals for the District of Columbia Circuit, decided June 9, 2015;

(b) Docket No EPA-HQ-OAR-201 3-0602; RIN 2060-AR33, a request for administrative stay of the Clean Power Plan filed with EPA on August 5,2015; and

(c) Case No. 15-1277 styled "In re: State of West Virginia, et al.," before the United States Court of Appeals for the District of Columbia Circuit, decided September 9, 2015".

Response: Enclosed please find a copy of Article VII, Section 1 of the West Virginia Constitution, the opinion of the Supreme Court of Appeals of West Virginia in *State ex rel. McGraw v. Burton*, 212 W. Va. 23, 569 S.E.2d 99 (2002), and West Virginia Code § 5-3-2. As the Supreme Court of Appeals explained in *Burton*, there is a difference between cases in which the Attorney General represents a State agency or office and those where he represents "the State itself." *Burton*, 212 W. Va. 23, 31, 569 S.E.2d 99, 107. The Office of the Attorney General of the State of West Virginia has a core and inviolable authority under the West Virginia Constitution to serve as the "constitutional officer who is directly elected by and accountable to the people [and who] may express his legal view on matters of State legal policy generally and particularly before tribunals where the State is a party." *Id.* at 39-40, 569 S.E.2d at 115-16. That authority includes the right to appear "on behalf of the State in all proceedings where the interest of the State or a State entity is at issue, to assert the Attorney General's view of the law on behalf of the State." *Id.* at 41, 569 S.E.2d at 117. Even in matters where a State agency or officer takes a different view from the Attorney General, "the Attorney General's intervenor standing permits the presentation of the Attorney General's view." *Id.* at 41 n.27, 569 S.E.2d at 117 n.27. Consistent with that independent and inherent constitutional authority, West Virginia Code § 5-3-2 provides that "[t]he attorney general shall appear as counsel for the state in all causes pending in the supreme court of appeals, or in any federal court, in which the state is interested."

Pursuant to W.Va. Code § 29B-1-3, I am required to inform you that having now replied to your request, no further action is required and our response to your request is at an end. In addition, I am required to inform you that if you are dissatisfied with this response, you have the right to institute proceedings for declaratory or injunctive relief in the Circuit Court of Kanawha County.

Sincerely,

Steven A. Travis
Assistant Attorney General

KeyCite Yellow Flag - Negative Treatment
Proposed Legislation

West's Annotated Code of West Virginia
The Constitution of West Virginia (1872)
Article VII

Const. Art. 7, § 1

§ 1. Executive department

Currentness

The executive department shall consist of a governor, secretary of state, auditor, treasurer, commissioner of agriculture and attorney general, who shall be ex officio reporter of the court of appeals. Their terms of office shall be four years and shall commence on the first Monday after the second Wednesday of January next after their election. They shall reside at the seat of government during their terms of office, keep there the public records, books and papers pertaining to their respective offices and shall perform such duties as may be prescribed by law.

Credits

Acts 1933, 1st Ex. Sess. H.J.R. 7 and Acts 1933, 1st Ex. Sess., c. 30, ratified Nov. 6, 1934; Acts 1957, S.J.R. 1 and Acts 1957, c. 19, ratified Nov. 4, 1958.

Notes of Decisions (34)

Const. Art. 7, § 1, WV CONST Art. 7, § 1
Current with laws of the 2015 Regular Session

End of Document

Governor Tomblin Announces West Virginia Will Take Steps to Submit Plan to Comply with Clean Power Plan

10/27/2015

CHARLESTON, W.Va. (October 27, 2015) – Gov. Earl Ray Tomblin issued the following statement regarding West Virginia's plan to comply with the Environmental Protection Agency's (EPA) Clean Power Plan during the annual Governor's Energy Summit:

"As required by new legislation passed by the Legislature this year, the state Department of Environmental Protection has already initiated its feasibility study to determine what options may be available for West Virginia to meet these new standards. Ultimately, any plan submitted by West Virginia will require the approval of the Legislature before being submitted to the EPA.

"While I believe there are significant questions regarding the legality of the Clean Power Plan, these new rules have been put into place by the federal regulatory agency. Until a final legal decision has been made, we cannot afford to ignore them. If we do not submit a plan, our state may be required to implement a plan designed by the EPA. If we can demonstrate that we put a lot of time and effort into developing a plan for West Virginia, we may have a better chance of lessening the harmful impacts these regulations could have on our miners, their families and communities.

"I'd prefer we start working this now so that when the time comes, we have an initial plan in place. By submitting this initial proposal, we'll have two additional years and the flexibility we need to complete a final plan. If the EPA feels the state's plan does not meet its standards, we have at least developed a starting point that gives us the opportunity to work toward a proposal that balances the environmental protection we all support with the economic growth and development we must maintain."

Contact Information

304-558-4977

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Clean Power Plan Comments and Information for Study Sought

10/16/2015
CHARLESTON, W.Va. – The West Virginia Department of Environmental Protection is working on a feasibility study related to the U.S. EPA's Clean Power Plan for Existing Power Plants and is seeking public comment and data. That information will be accepted through the end of the year.

The study concerns the feasibility of developing a state plan for the regulation of emissions of carbon dioxide, a greenhouse gas, in order to comply with the requirements in the EPA's rule. This feasibility study, which is being conducted with participation from Marshall University researchers, is mandated by House Bill 2004, which passed the Legislature earlier this year. The study requires a comprehensive analysis of the potential effects the rule, which is 111 (d) of the Clean Air Act, on the state, its people and its economy. Those who wish to supply comments and information for use in this study should consult the legislation, which can be read in its entirety here.

The DEP has 180 days from the effective date of the 111 (d) rule to conduct this study. That clock starts when the rule is published in the federal register, which is expected to occur later this month.

If development of a State Implementation Plan (SIP) is deemed feasible, the next step would be to develop such a plan and submit it to the West Virginia Legislature for approval prior to submittal of the plan to the EPA. States must – by Sept. 6, 2016 – either submit a final SIP or file an initial plan with an extension request. Final completed state plans must be submitted no later than Sept. 6, 2018.

The EPA has said it will impose a Federal Implementation Plan (FIP) on states that do not submit state plans. The proposed FIP is not anticipated to be finalized until after the DEP's report to the state Legislature is due. The EPA also has not yet finalized model state plans.

Anyone with data that could be useful in the development of the feasibility study and/or state plan, or who would like to provide comments about the Clean Power Plan in general, is invited to share that information with the DEP. Comments can be emailed to dep.comments@wv.gov, with "Clean Power Plan Study Comments" in the subject line, by Dec. 31, 2015. Information can also be mailed by that date to:
Clean Power Plan Study Comments
West Virginia DEP
601 57th Street SE
Charleston, WV 25304

Formal requests for data from electric utilities, various consumer, citizen and industry groups and government entities have also been sent out.

The goal of the Clean Power Plan is to – by 2030 – cut carbon pollution from the power sector 32 percent below 2005 levels. For more information on the EPA's rule, the DEP's feasibility study and important dates related to both, click here.

For more DEP news and information, go to www.dep.wv.gov. Also, be sure to connect with the agency on all social media platforms. Follow @DEPWV on Twitter and find us on YouTube by searching "Environment Matters." For specific information about our REAP (Rehabilitation Environmental Action Plan), West Virginia Project WET (Water Education for Teachers), West Virginia Watershed Improvement Branch, Youth Environmental Program and Human Resources initiatives, connect on Facebook.

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Contact:

Kelley Gillenwater
304-926-0440
kelley.j.gillenwater@wv.gov

Appalachian Mountaintop Mining Particulate Matter Induces Neoplastic Transformation of Human Bronchial Epithelial Cells and Promotes Tumor Formation

Sudjit Luanpitpong,[*,†,‡,§] Michael Chen,[†,‡] Travis Knuckles,[¶] Sijin Wen,[‖] Juhua Luo,[⊪] Emily Ellis,[#] Michael Hendryx,[⊪] and Yon Rojanasakul[†,‡]

[†]Department of Pharmaceutical Sciences, West Virginia University, Morgantown, WV 26506, USA; [‡]Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV 26506, USA; [§]Siriraj Center of Excellence for Stem Cell Research, Mahidol University, Bangkok 10700, Thailand; [¶]Center for Cardiovascular and Respiratory Science, West Virginia University, Morgantown, WV 26506, USA; [‖]Department of Biostatistics, West Virginia University, Morgantown, WV 26506, USA; [⊪]School of Public Health, Indiana University, Bloomington, IN 47405, USA; [#]Animal Models and Imaging Facility, West Virginia University, Morgantown, WV 26506, USA.

The project described was supported by the National Institute of General Medical Sciences, U54GM104942.

***CORRESPONDENCE:** Sudjit Luanpitpong, West Virginia University, Health Sciences Center, Morgantown, WV 26506. Tel: 304 293 1483; Fax: 304 293 2576; Email: suidjit@gmail.com

ABSTRACT

Epidemiological studies suggest that living near mountaintop coal mining (MTM) activities is one of the contributing factors for high lung cancer incidence. The purpose of this study was to investigate the long-term carcinogenic potential of MTM particulate matter (PM_{MTM}) exposure on human bronchial epithelial cells. Our results show that chronic exposure (3 months) to non-cytotoxic, physiological relevant concentration (1 μg/mL) of PM_{MTM}, but not control particle PM_{CON}, induced neoplastic transformation, accelerated cell proliferation and enhanced cell migration of the exposed lung cells. Xenograft transplantation of the PM_{MTM}-exposed cells in mice caused no apparent tumor formation, but promoted tumor growth of human lung carcinoma H460 cells, suggesting the tumor promoting effect of PM_{MTM}. Chronic exposure to the main inorganic chemical constituent of PM_{MTM}, molybdenum but not silica, similarly induced cell transformation and tumor promotion, suggesting the contribution of molybdenum, at least in part, in the PM_{MTM} effects. These results provide new evidence for the carcinogenic potential of PM_{MTM} and support further risk assessment and implementation of exposure control for PM_{MTM}.

KEYWORDS: particulate matter; mountaintop mining; neoplastic transformation; cell proliferation; lung cancer.

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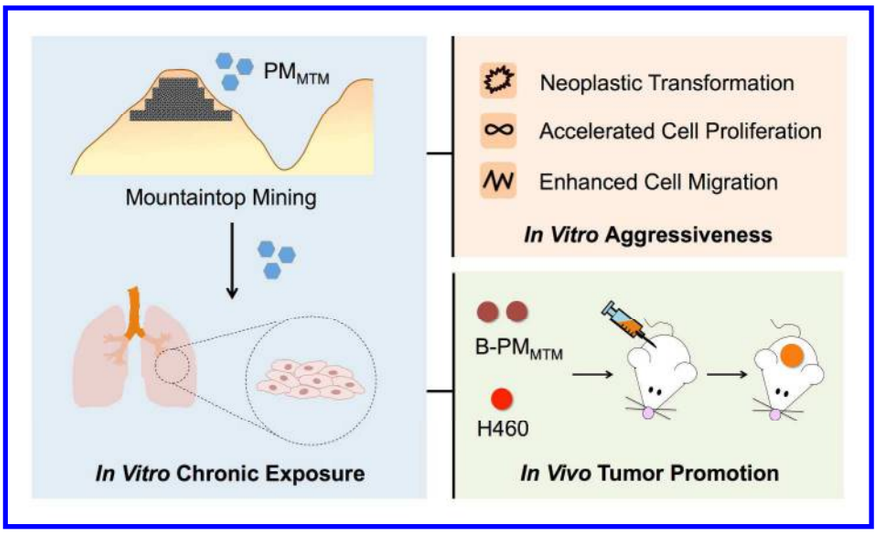

INTRODUCTION

Lung cancer is the leading cause of cancer-related death, and, after smoking, environmental and occupational exposure is a major cause.[1,2] The Appalachian Mountains stretch across 13 states of the United States from southern New York to northern Mississippi. Health disparities, most notably cancer incidence and mortality rate, are higher in the Appalachian region compared to the rest of the country.[3,4] Previous epidemiology studies demonstrated elevated lung cancer mortality in coal-mining areas of Appalachia,[5,6] suggesting that environmental contaminants from coal-mining activities may contribute to the increased lung cancer risk.

Mountaintop removal mining (MTM) is a major form of surface coal mining in Appalachia, especially in West Virginia and Kentucky.[7] In southern West Virginia, almost 40 million tons of coals were extracted by MTM in 2012.[8] Particulate matter (PM) is generated from these active MTM sites by blasting and combustion from heavy equipment, and may represent a potential toxicant that is elevated in ambient air.[9] The lungs are the primary target organ for these airborne MTM-derived PM (PM$_{MTM}$)

exposures.[10] To date, there have been no experimental reports on the potential carcinogenic effect of PM_{MTM}, either *in vitro* or *in vivo*. Since carcinogenesis is a multi-step process commonly associated with long-term exposure to carcinogens,[11,12] we studied the chronic effects of PM_{MTM} exposure on human bronchial epithelial cells, one of the major cellular targets of lung carcinogenesis. Such information is necessary to provide a scientific basis for the epidemiological finding on increased lung cancer mortality in the coal-mining areas of Appalachia.

In the present study, we chronically exposed human bronchial epithelial BEAS-2B cells to non-cytotoxic, physiologically relevant concentration of PM_{MTM} or control PM (PM_{CON}) over a 3-month period in culture. The exposed lung cells were then evaluated for their neoplastic transformation, proliferative and migratory properties *in vitro* and tumorigenicity *in vivo*. We also studied the effect of inorganic chemical constituents of PM_{MTM} by similarly exposing bronchial epithelial cells to silica (Si) and molybdenum (Mo), the main inorganic chemical constituents of PM_{MTM}. Our data indicate the cell-transforming and tumor-promoting effects of PM_{MTM}; thus supporting the prudent adoption of prevention strategies and implementation of exposure control for PM_{MTM}. The described chronic exposure model could further be used for mechanistic studies and risk assessment of PM_{MTM} which may not be feasible *in vivo*.

MATERIALS AND METHODS

A more detailed description of Materials and Methods used in this study is available as Supporting Information at http://pubs.acs.org/.

Cell Culture

Human bronchial epithelial BEAS-2B and non-small cell lung cancer H460 cells were obtained from American Type Culture Collection (ATCC; Manassas, VA) and were cultured as described previously.[13]

Collection of MTM and control particulate matters

Air samples were taken at two rural residential sites located within 1 mile of an active MTM site in Edwight, WV, USA. For control, air was similarly sampled from selected rural areas in Green Bank, WV, which does not have coal mining[14]. PM_{MTM} and PM_{CON} were collected on 5 μm-pore size PTFE fiber-backed filters (Whatman, Springfield Mill, UK) for 2-4 weeks. The filters were extracted according to the method previously described (see Supporting Information Table S1 for PM mass).[15] It is worth noting that this method of PM collection could not preserve the volatile organic compounds. Scanning Electron Microscope-Energy-Dispersive X-ray Spectroscopy (SEM-EDX), which was limited to the analysis of inorganic compounds, was further used to perform PM compositional analysis (RTI International, Research Triangle Park, NC). In comparison with PM_{CON}, Si and Mo were found to be the main inorganic chemical elements in PM_{MTM} with the % weight average of 48.15 ± 26.91% and 28.90 ± 4.16% respectively for Si and Mo *vs.* 23.75 ± 15.07% and 0.00 ± 0.00% of the elements in PM_{CON} (see Table S2 for analysis of organic elements).

Cytotoxicity Assay

Cell viability was determined by MTT assay as described previously.[16] All particles were suspended in phosphate buffer saline (PBS) containing 5% bovine serum albumin (BSA) and were lightly sonicated prior to use to disperse the particles. The absorbance ratio of

MTT formazan product of treated and non-treated cells was calculated and presented as relative cell viability.

Chronic Particle Exposure

Subconfluent cultures of bronchial epithelial BEAS-2B cells were continuously exposed to non-cytotoxic concentration (1 μg/mL) of PM_{MTM} or PM_{CON} in 6-well plates for 3 months and were passaged biweekly. PM_{MTM}- and PM_{CON}-exposed BEAS-2B cells were designated as B-PM_{MTM} and B-PM_{CON} cells. Parallel culture grown with the same background level of dispersant provided a passage-matched control (B-NTX cells). To study the effect of PM_{MTM} inorganic chemical elements, cells were similarly exposed to a non-cytotoxic concentration (1 μg/mL) of Si or Mo for 3 months (designated as B-Si and B-Mo cells). All cells were cultured in complete medium (without treatment) for at least 10 passages prior to experiments to rule out any reversible effects.

Dosage Calculation and Human Extrapolation

PM exposure dose of 1 μg/mL in the 6-well plates (growth area ~ 10 cm^2) at the total volume of 1 mL corresponds to the surface area dose of 0.1 μg/cm^2. Based on the reported rat lung surface area of 5000 cm^2,[17] this exposure dose is equivalent to a bolus exposure of PM at 0.5 mg in the rats, which was previously shown to induce pathological changes.[18] Assuming the pulmonary surface area in humans of 100 m^2, the human burden is equal to 100 mg/lung. Considering a respiratory deposition of ~40%[14] and an adult inhalation rate of ~16 m^3/day,[19] the experimental dose could be reached within 8.5 years of human inhalation exposure at 5 μg/m^3 (average total PM mass concentration in Edwight and Green Bank, WV).[14]

Soft Agar Colony Formation Assay

The chronic exposed cells at 3×10^4 cells per 24-well plate were mixed with culture medium containing 0.5% agar. The resulting cell suspensions were immediately plated onto dishes coated with 0.5% agar in culture medium. After 2 weeks, colonies larger than 50 μm in diameter were scored as positive for growth.[20]

Cell Counting

The exposed cells (3×10^4 cells) were seeded in 24-well plates and cultured in complete medium. The cells were stained with 0.4% trypan blue (Invitrogen) (to indicate dead cells) and healthy cell number was scored using Countess® automated cell counter (Invitrogen) at 2 and 5 days.

Proliferative Index

The chronic exposed cells (2×10^6 cells) were labeled with CellVue® Claret Far Red Fluorescent Cell Linker (Sigma) according to the manufacturer's protocol. After 4 days of culture, proliferative index was determined based on far red fluorescence intensity using FSC Express 4 Flow Cytometry software (De Novo Software, Los Angeles, CA).

Cell Cycle Analysis

The chronic exposed cells were serum starved for 12 hours and incubated in the complete medium for 8 hours. The cells were then stained with 20 μg/mL PI and the percentage of cells in different phases of cell cycle was determined by FSC Express 4 software.

Migration Assay

Cell migration was determined by wound healing assay as previously described.[16] Briefly, a monolayer of chronic exposed cells was cultured in 24-well plate and a wound space was created with a 1-mm width tip. The cell monolayers were incubated in complete medium and allowed to migrate for 24 hours.

Xenograft Mouse Model

Animal care and experimental procedure described in this study were in accordance with the Guidelines for Animal Experiments at West Virginia University (IACUC #12-0502). Immunodeficient NOD/SCID gamma mice, strain NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG; Jackson Laboratory, Bar Harbor, ME), were maintained under pathogen-free conditions within the institutional animal facility.[21,22] Mice were injected subcutaneously (SC) with 3×10^5 luciferase (Luc2; Capital Biosciences, Rockville, MD)-labeled lung cancer H460 cells and 6×10^5 PM-exposed cells (1:2 ratio) suspended in 100 µL of ExtraCel® hydrogel (Advanced BioMatrix, San Diego, CA). Tumor growth of luciferase-labeled cells was monitored weekly using IVIS® bioimaging (Perkin Elmer, Waltham, MA). At the end of experiments, mice were euthanized and SC tumors were dissected and weighted. Metastasis of tumor cells to neighbor tissues was analyzed by IVIS® imaging after removal of primary SC tumors.

Statistical Analysis

The data represent means ± SD from three or more independent experiments as indicated. Statistical analysis was performed by Student's t test at a significance level of $P < 0.05$.

RESULTS

Effects of PM_{MTM} on Cytotoxicity

The purpose of this study was to establish an experimental human lung cell model for PM_{MTM} carcinogenesis studies that would allow further molecular and cellular mechanistic studies underlying cancer-like phenotypes. We first characterized the acute cytotoxic effect of PMs to determine their non-cytotoxic concentrations for subsequent long-term exposure studies. Human bronchial epithelial cells were exposed to various concentrations (0.1-10 μg/mL) of PM_{CON} and PM_{MTM} for 48 hours and cell viability was determined by MTT assay. The results showed that none of the PM_{CON} and PM_{MTM} treatments caused a significant effect on cell viability as compared to non-treated (NTX) control (Figure 1*A*). We similarly tested the dose effect of inorganic chemical elements of PM_{MTM} (Si and Mo) on cell viability. The results similarly showed the non-cytotoxic effect of Si and Mo at the treatment doses of 0.1-10 μg/mL (Figure 1*B*). As we observed a slight increase in cell viability (proliferation), albeit not significant, at the high dose of PM_{MTM} (10 μg/mL), we used a lower dose (1 μg/mL) in our subsequent chronic exposure studies.

Chronic PM_{MTM} Exposure Induces Neoplastic Transformation

To mimic the long-term carcinogenic process, cells were chronically exposed to a non-cytotoxic concentration of PMs at 1 μg/mL (0.1 μg/cm^2 surface area dose) and passaged biweekly. This surface area dose mimics the *in vivo* dose in rodents of 0.5 mg or approximately 8.5 years of human inhalation exposure as described in Materials and Methods. In this study, the cells were exposed to PM_{MTM}, PM_{CON}, or left untreated for 3 months (Figure 2*A*), after which they were grown in complete medium (without treatment) for at least 10 passages and examined for anchorage-independent growth by soft-agar colony formation assay, which is one of the most stringent indicators of neoplastic transformation.[23] To determine the key inorganic constituents of PM_{MTM} that

may contribute to its pathological effect, cells were similarly exposed to Si and Mo, and analyzed for cell transformation. Figure 2*B* and *C* show that as compared to particle-control B-PM_{CON} cells, the B-PM_{MTM} and B-Mo cells formed larger and greater numbers of colony, whereas the B-Si cells exhibited a similar colony forming activity. These results indicate the neoplastic transformation of B-PM_{MTM} and B-Mo cells.

Chronic PM_{MTM} Exposure Alters Cell Growth Characteristic

Excessive cell growth is one of the carcinogenic properties of malignant cells.[24,25] To determine whether chronic PM exposure affects cell growth characteristic, the exposed cells were analyzed for cell proliferation by direct cell counting and dye-based assays. Figure 3*A* shows that the B-PM_{MTM} and B-Mo cells exhibited a significantly higher proliferation rate than the B-PM_{CON} cells, which grew at a similar rate as the B-NTX and B-Si cells. To confirm this result which was based on direct cell counting assay, the cells were stained with membrane dye CellVue® Claret and their proliferative index was determined by flow cytometry. This dye-based assay measures cell proliferation based on the principle of dye dilution upon cell division. Consistent with the direct cell counting result, the dye-based assay indicated a higher proliferative index of B-PM_{MTM} and B-Mo cells compared to B-PM_{CON} and B-Si cells (Figure 3*B*). Analysis of cellular fluorescence intensity further indicated the division of parental cells with the observed seventh generation of daughter cells only in the B-PM_{MTM} and B-Mo cells (Figure 3*C*), thus substantiating the above finding.

To delineate the mechanism of PM_{MTM}-induced cell proliferation, we investigated the cell cycle progression of synchronized B-PM_{MTM}, B-PM_{CON}, B-Si, and B-Mo cells by flow cytometry using propidium iodide (PI) DNA staining assay. As depicted in Figure 4*A*, a higher percentage of B-PM_{MTM} cells compared to B-PM_{CON} cells entered the S

phase (~80% *vs.* 50%) and reached the G2/M transition phase (~5% *vs.* 1%), whereas the B-Mo cells had a significant portion in the G2/M phase (~10%). These results indicated the promotion of S phase entry by chronic PM_{MTM} exposure and the transition to G2/M phase by chronic Mo exposure.

Chronic PM_{MTM} Exposure Promotes Cell Migration

The aggressive behavior of PM_{MTM}-exposed cells was examined by assessing their migratory activity, which is a key determinant of tumor invasion and progression.[26,27] Cell migration was determined by scratch or wound healing assay. At 24 hours after the scratch, B-PM_{MTM} and B-Mo cells showed a significantly higher motility rate towards the wound compared to B-NTX, B-PM_{CON} and B-Si cells, as judged by their greater wound closure (Figure 5*A* and *B*). These results indicate the induction of aggressive cell behavior by chronic exposure to PM_{MTM} and Mo.

Tumorigenicity Assessment of PM_{MTM} Cells in Mice

Carcinogenesis is a multistep sequential process consisting of three major stages, namely initiation, promotion, and progression.[12,28,29] Certain carcinogens can act in one or all of these stages, which results in neoplastic transformation and tumor development.[30] Having demonstrated the neoplastic transformation of B-PM_{MTM} cells, we next assessed their tumorigenic potential *in vivo*. The B-PM_{MTM} cells and their control B-PM_{CON} cells as well as B-NTX, B-Mo and B-Si cells (1×10^6) were injected into NSG mice subcutaneously and tumor formation was determined over time. No tumor formation was observed with any of the above treatments including those injected with the neoplastic B-PM_{MTM} and B-Mo cells (Figure 6*A*), indicating their inherent non-tumorigenicity. To test whether these cells might possess tumor-promoting activity, we co-injected the B-

234 PM_{MTM}, B-PM_{CON}, B-Si or B-Mo cells (6×10^5) with tumorigenic human lung cancer
235 H460 cells (3×10^5), which have been modified to express luciferase to aid quantitation of
236 tumor formation in mice by bioluminescence imaging (Figure 6*B*). Tumor luminescence
237 signals were quantified over time and normalized to their initial signal at the time of
238 inoculation (day 1). At 1 week post-injection, tumor luminescence was higher in mice
239 bearing the H460 cells with B-PM_{MTM}, B-Mo or B-Si cells as compared to the mice
240 bearing the H460 with B-PM_{CON} cells (Figure 6*C*). At 2 weeks post-injection, the tumor
241 luminescence intensity was high only in the mice injected with H460 cells and B-PM_{MTM}
242 or B-Mo cells, but not B-PM_{CON} or B-Si cells (Figure 6*D*). These results indicate the
243 tumor-promoting activity of B-PM_{MTM} and B-Mo cells.

244 At the end of the experiments (week 3), SC tumors were dissected and their
245 bioluminescence were determined and compared between groups (Figure 7*A*). Figure 7*B*
246 shows a stronger bioluminescence signal in tumors from H460 and B-PM_{MTM} or H460
247 and B-Mo cells, compared to those from H460 and B-PM_{CON} or H460 and B-Si cells.
248 Analysis of tumor weight of the samples further supported the tumor promoting role of
249 B-PM_{MTM} and B-Mo cells (Figure 7*C*). Interestingly, we observed notable tumor
250 bioluminescence signals in mice bearing H460 and B-PM_{MTM}, B-Si or B-Mo cells after
251 the dissection of SC tumors (Figure 7*A* and *D*), suggesting metastasis of tumor cells to
252 neighboring tissues and strengthening the important role of chronic PM_{MTM} and Mo
253 exposure in tumor promotion.

254

255 **DISCUSSION**

256 A growing body of evidence links living in proximity to MTM activities to greater risk of
257 serious health consequences, including significantly higher reports of cancer.[31] The MTM
258 operation uses explosive and excavation equipment to remove vegetation, rock, and dirt

from mountaintops to expose coal seams, and thus, it consists of active areas of blasting, crushing, and grinding.[7] MTM activities result in the production of atmospheric PM (PM_{MTM}) that might be associated with human health effects. Currently, the direct relationship between chronic pulmonary exposure to PM_{MTM} and lung cancer risk has not been investigated. In this study, we reported a combined *in vitro-in vivo* model for PM_{MTM} lung carcinogenesis studies using chronically exposed human bronchial epithelial BEAS-2B cells and a mouse xenograft model. Bronchial epithelial cells were chosen in this study as they are one of the key targets for lung carcinogenesis. Airway epithelium lines the body's first physiological barrier to inhaled PM and the particles in range of 1-5 μm generally deposited in the tracheobronchial region of the airways – such deposition appears to be a close correlation with the incidence of primary cancer sites.[32]

Anchorage-independent growth has been well correlated with the tumorigenicity and invasiveness of several cancer cell types.[24] Colony formation under soft agar assay, the gold standard test to evaluate the ability of cells to undergo anchorage-independent growth, is therefore the most stringent indicator for neoplastic transformation. We showed that chronic PM_{MTM}-exposed B-PM_{MTM} cells induced larger number and size of colonies as compared to chronic PM_{CON}-exposed B-PM_{CON} cells and passage-matched control B-NTX cells (Figure 2). It has previously been reported that BEAS-2B cells might undergo squamous differentiation in the presence of serum.[33,34] However, since B-PM_{CON} and B-NTX cells showed no phenotypic changes or neoplastic behavior under the culture condition, it can be concluded that the B-PM_{MTM} cells, not B-PM_{CON} and B-NTX cells, have undergone differentiation and show altered phenotype due to continued exposure. In order to delineate the chemical effects of PM_{MTM} inorganic elements, bronchial epithelial cells were similarly exposed to Si and Mo and neoplastic transformation was observed in the Mo-exposed B-Mo cells, but not Si-exposed B-Si

cells. Given that the magnitude of Mo effect was similar to the PM_{MTM}, despite its higher concentration than those presented in PM_{MTM}, it is likely that: (i) some other elements could be involved in the PM_{MTM} effect; and (ii) such effect arose from the synergistic effect of more than one component, e.g. Si and Mo.

Various carcinogenic properties representing the hallmarks of malignant cells were further assessed in this study. The transformed B-PM_{MTM} and B-Mo cells demonstrated excessive cell growth and altered cell cycle (Figure 3-4). To our knowledge, this is the first demonstration of the induction of cell proliferation by chronic low-dose PM exposure, although the inhibition of cell proliferation[35,36] and induction of cytotoxicity[37,38] by acute high-dose PMs from urban and industrial areas have previously been demonstrated. Interestingly, B-PM_{MTM} and B-Mo cells promoted cell cycle at different phases, possibly due to: (i) the low content of Mo present in the PM_{MTM} that might not be sufficient to drive the cells to G2/M phase; and (ii) the PM_{MTM} proliferative effect was by components other than Mo or a synergistic effect of more than one component. Cell motility was also shown to increase significantly in the B-PM_{MTM} and B-Mo cells as compared to B-NTX cells (Figure 5), thus indicating their aggressive behaviors, which could be important in tumor progression.

The potential role of PM_{MTM} in lung carcinogenesis was further evaluated *in vivo* using a mouse xenograph model. Our results demonstrated that B-PM_{MTM} cells, although did not directly induce tumors in mice, promoted tumor formation and metastasis of human lung cancer H460 cells (Figure 6-7). The limitation of our *in vivo* study is the relatively small number of animals per group that, with the high individual biological variation, we could not obtain statistical power > 80%, although we did achieve statistical significance ($P < 0.05$). These results however are in good agreement with previous reports showing the hypermethylation of tumor suppressor p16 by PMs from urban areas

which could lead to cancer development[39] and the induction of lung carcinoma by Mo inhalation exposure in mice.[40]

Taken together, the present study demonstrated that chronic exposure to PM_{MTM} induced neoplastic transformation of human bronchial epithelial cells with cancer-like properties. While the data did not indicate tumor initiation by PM_{MTM}, the lung tumor promotion and progression by PM_{MTM} are a health concern as a cancer promoter. Our finding strengthens previous epidemiological studies linking MTM to increased incidence of lung cancer[5,6,41] and supports prudent adoption of prevention strategies and exposure control for PM_{MTM}. As more than 60,000 cancer cases has been estimated to correlate with MTM activities in West Virginia,[31] this finding on the cancer promoting effect of PM_{MTM} and related epidemiological data are crucial to raise public health awareness to reduce cancer risk. Our study also suggested that Mo could be one of the key inorganic elements responsible for the cancer promoting effect of PM_{MTM}, although we could not rule out the involvement of organic elements as well as the synergistic effect of more than one element in the process. Finally, the chronic exposure model described in this study may be useful in further mechanistic studies and risk assessment of PM_{MTM} pathogenicity.

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SUPPORTING INFORMATION

Information includes the PM mass used in this study (Table S1), PM_{MTM} analysis of organic/elemental carbon, metals and sulphate (Table S2) and Supplementary Materials and Methods. This information is available free of charge via the Internet at http://pubs.acs.org/.

ABBREVIATIONS

MTM, mountaintop coal mining; PM, particulate matter; PM_{MTM}, MTM-derived PM; PM_{CON}, control PM; Si, silica; Mo, molybdenum; NTX, non-treatment; B-NTX, passage-matched control bronchial epithelial cells; B-PM_{CON}, chronic PM_{CON}-exposed bronchial epithelial cells; B-PM_{MTM}, chronic PM_{MTM}-exposed bronchial epithelial cells; B-Si, chronic Si-exposed bronchial epithelial cells; B-Mo, chronic Mo-exposed bronchial epithelial cells; NSG mice, NOD/SCID gamma mice; SC, subcutaneous.

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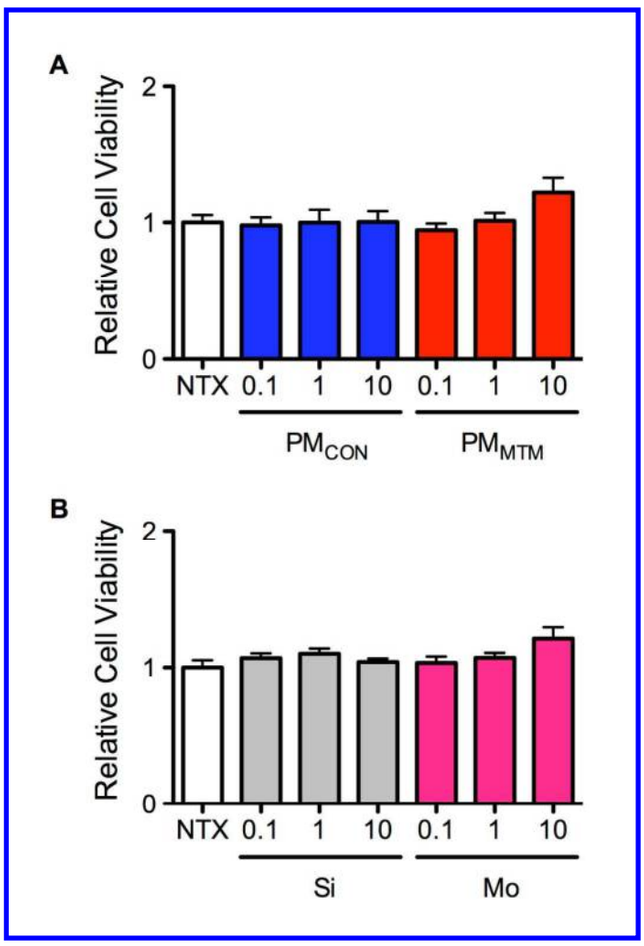
480 **FIGURES AND LEGENDS**



481

482 **Figure 1.** Effect of acute PM_{MTM} exposure on cytotoxicity of human bronchial epithelial
483 cells. (*A*) Subconfluent monolayers of BEAS-2B cells were left untreated (non-treatment,
484 NTX) or treated with various concentrations (0.1-10 μg/mL) of PM_{MTM} and PM_{CON} for 48
485 hours and analyzed for cell viability using MTT assay. (*B*) Cells were treated with Si or
486 Mo at the same concentration range and analyzed for cell viability after 48 hours by MTT
487 assay. Data are mean ± SD (n = 4).
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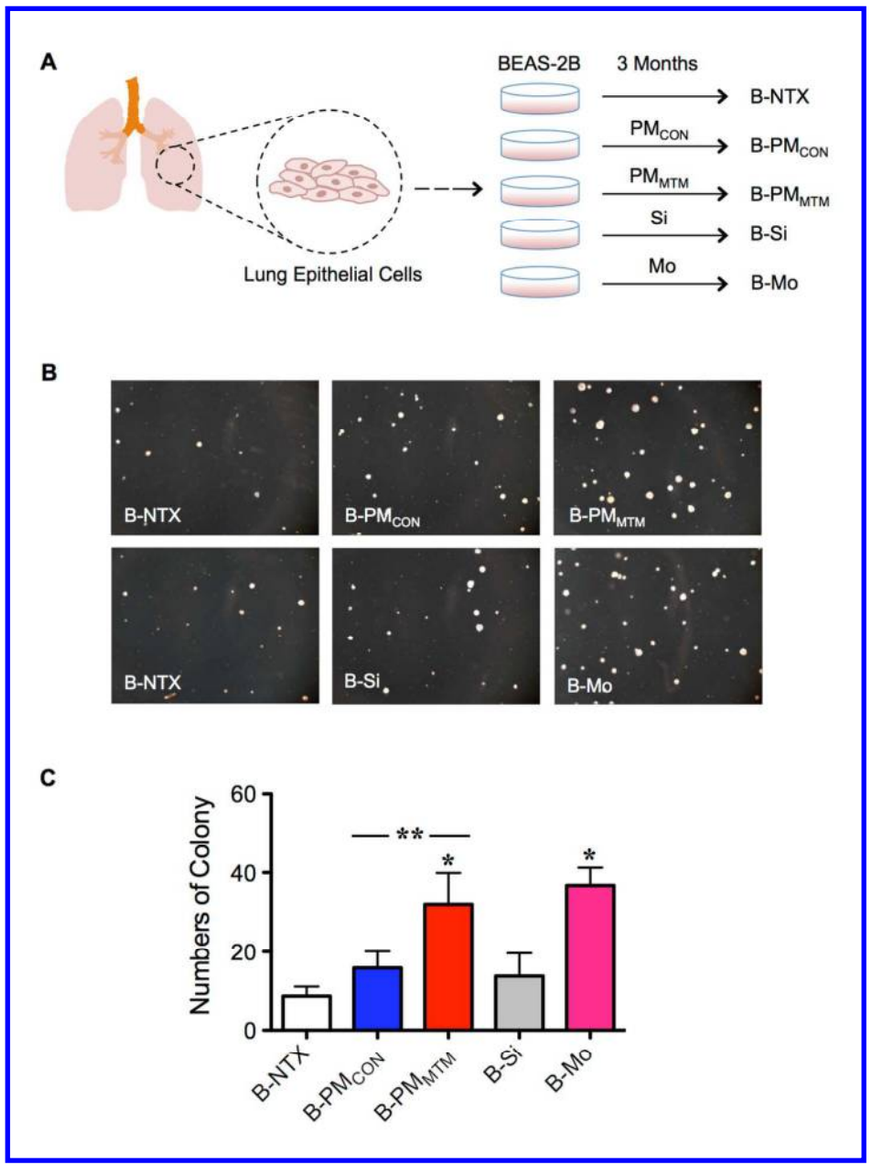


Figure 2. Chronic exposure to PM_{MTM} induces neoplastic transformation of human bronchial epithelial cells. (*A*) Schematic representation of chronic exposure model. BEAS-2B cells were continuously exposed to non-cytotoxic concentration (1 μg/mL) of PM_{CON}, PM_{MTM}, Si and Mo for 3 months and designated as B-PM_{CON}, B-PM_{MTM}, B-Si and B-Mo cells, respectively. BEAS-2B cells maintained in culture without particle exposure (B-NTX) served as passage control cells. (*B*, *C*) Cells were seeded on 0.5% agar plates and after 2 weeks they were visualized under a phase contrast microscope. (C) Quantification of large colonies (> 50 μm in diameter). Data are mean ± SD ($n = 4$). *$P <$ 0.05 (power > 95%) *vs.* passage control B-NTX cells. **$P < 0.05$ (power > 80%) *vs.* B-PM_{CON} cells.

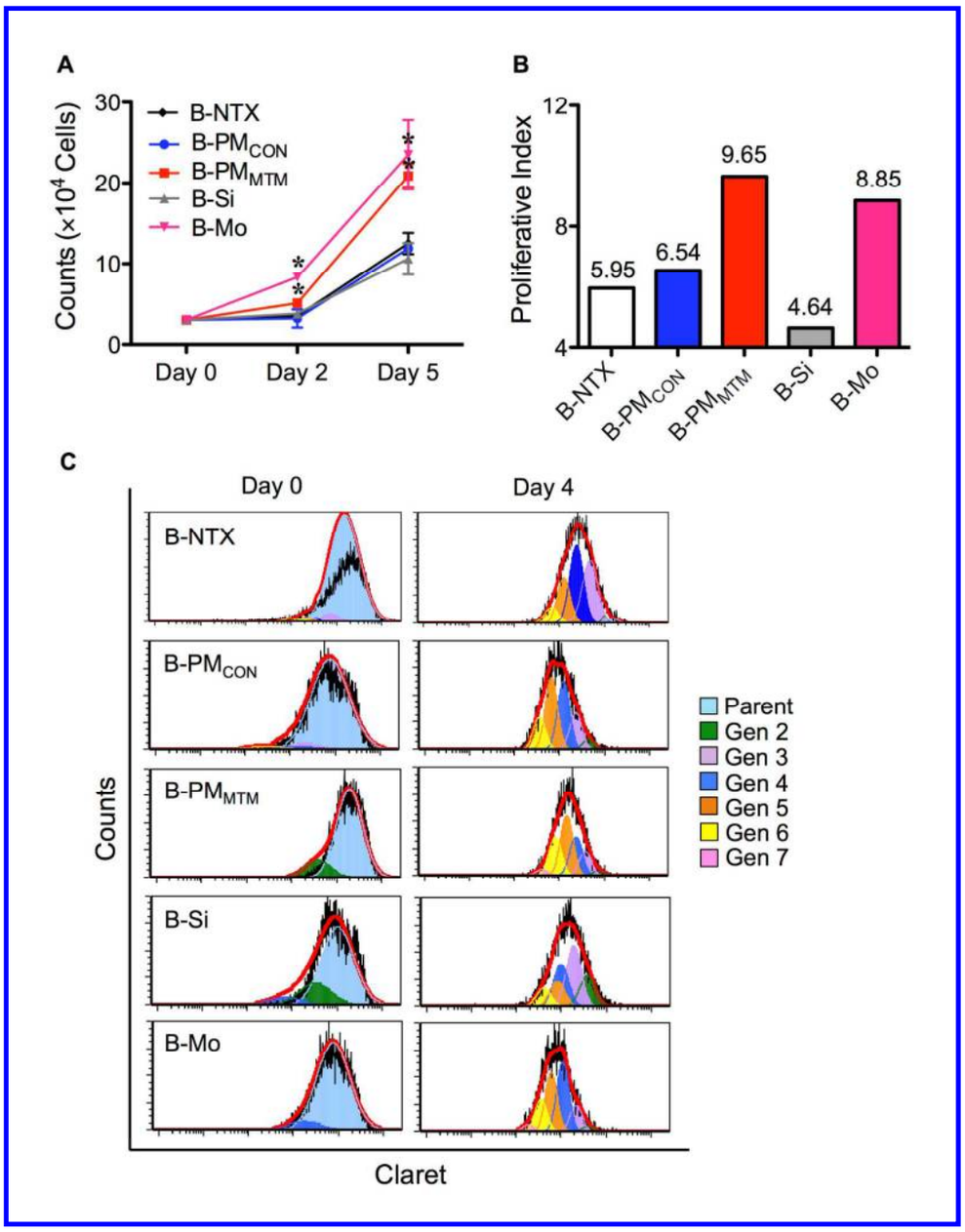


Figure 3. Chronic PM_{MTM} exposure accelerates proliferation of human bronchial epithelial cells. (*A*) B-PM_{CON}, B-PM_{MTM}, B-Si, B-Mo and B-NTX cells were plated in 24-well plates at the density of 3×10^4 cells in growth medium. After 2 and 5 days, the cells were counted using an automated cell counter. Data are mean ± SD (n = 3). *P < 0.05 (power > 95%) *vs.* passage control B-NTX cells. (*B*) Cells were labeled with membrane dye CellVue® Claret. After 4 days of culture, cellular fluorescence intensity was determined by flow cytometry and proliferative index was calculated. (*C*) Representative flow cytometric histograms from three independent experiments showing brightly stained parental cells and weaker stained daughter cells.

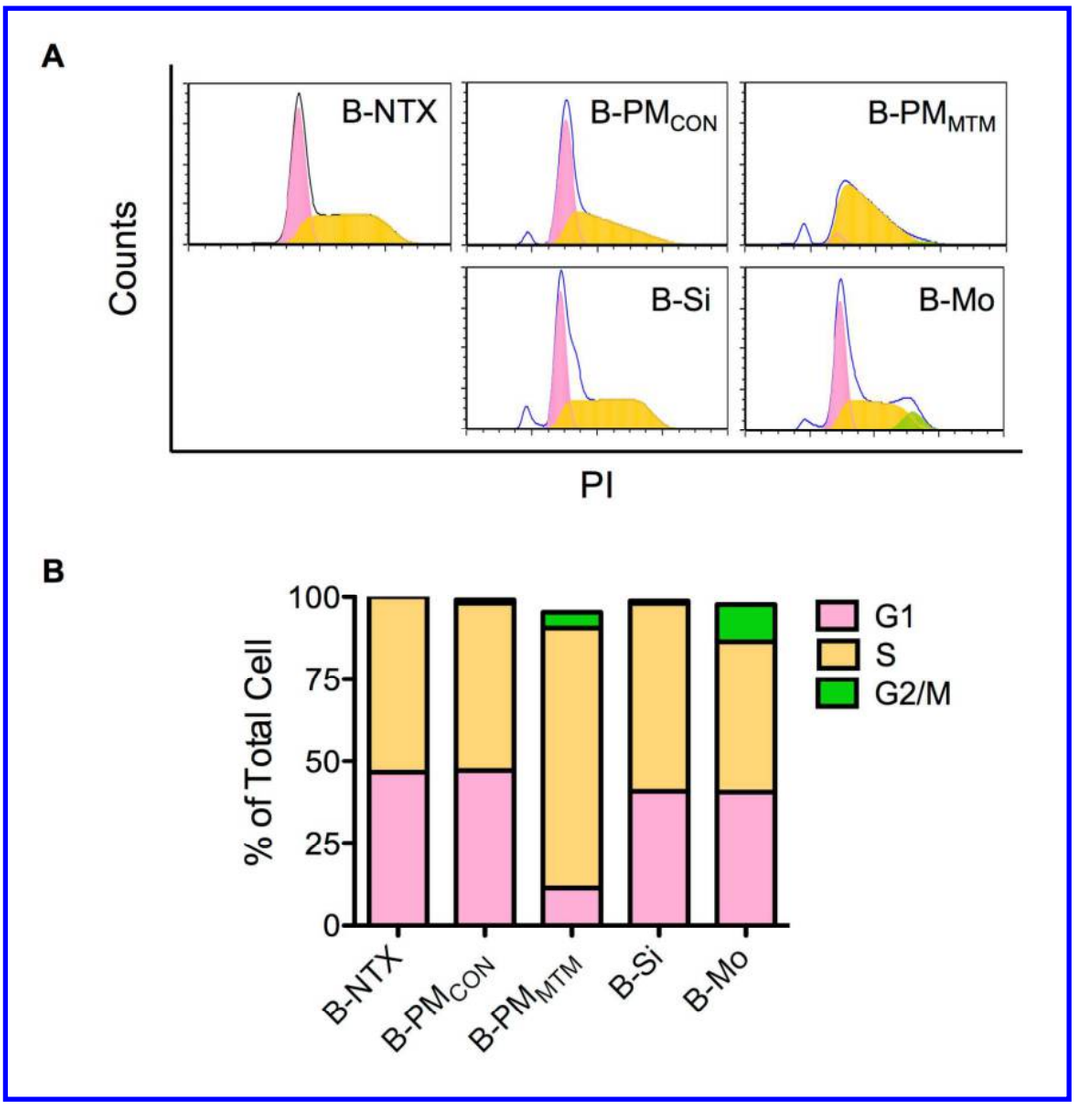


Figure 4. Chronic PM_{MTM} exposure alters cell cycle of human bronchial epithelial cells. (*A*) B-NTX, B-PM_{CON}, B-PM_{MTM}, B-Si and B-Mo cells were serum starved for 12 hours to synchronize their cell cycle. They were then cultured in a complete medium for 8 hours and analyzed for their cell cycle by flow cytometry. Representative histograms from three independent experiments were shown. (*B*) Plots are percentages of total cells (50,000 events) in each phase of the cell cycle (G1, S and G2/M).

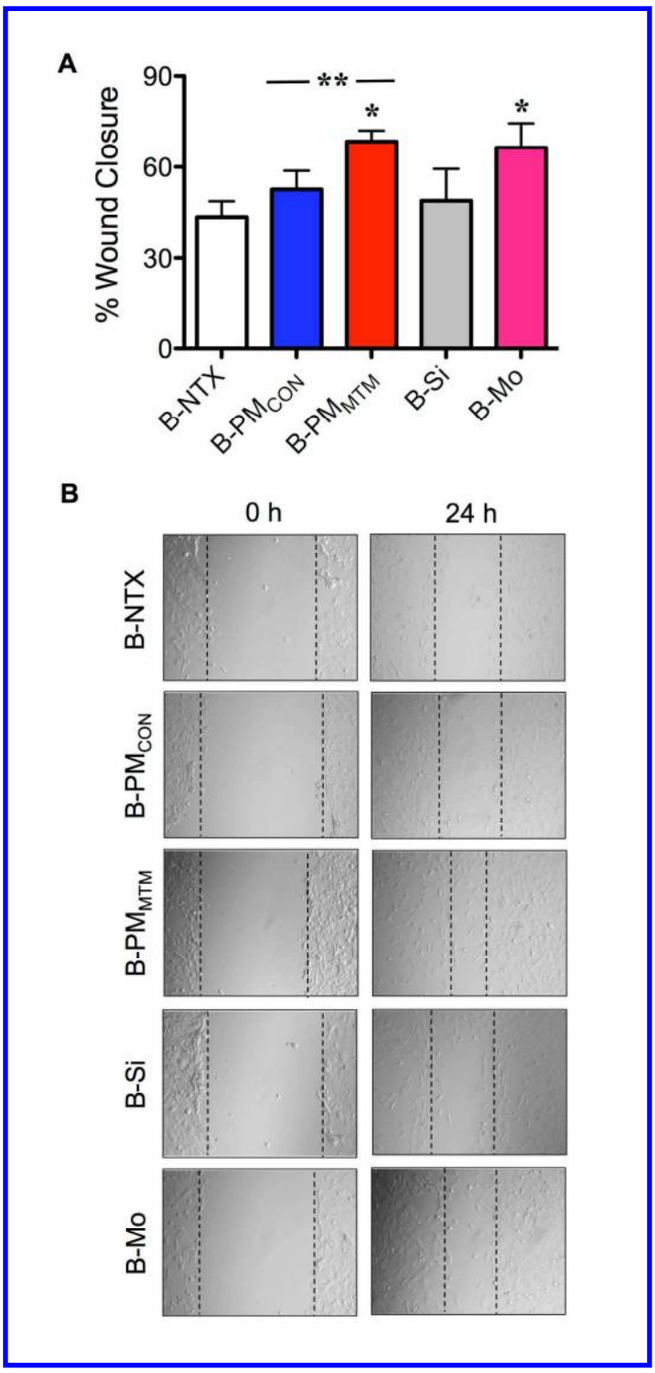
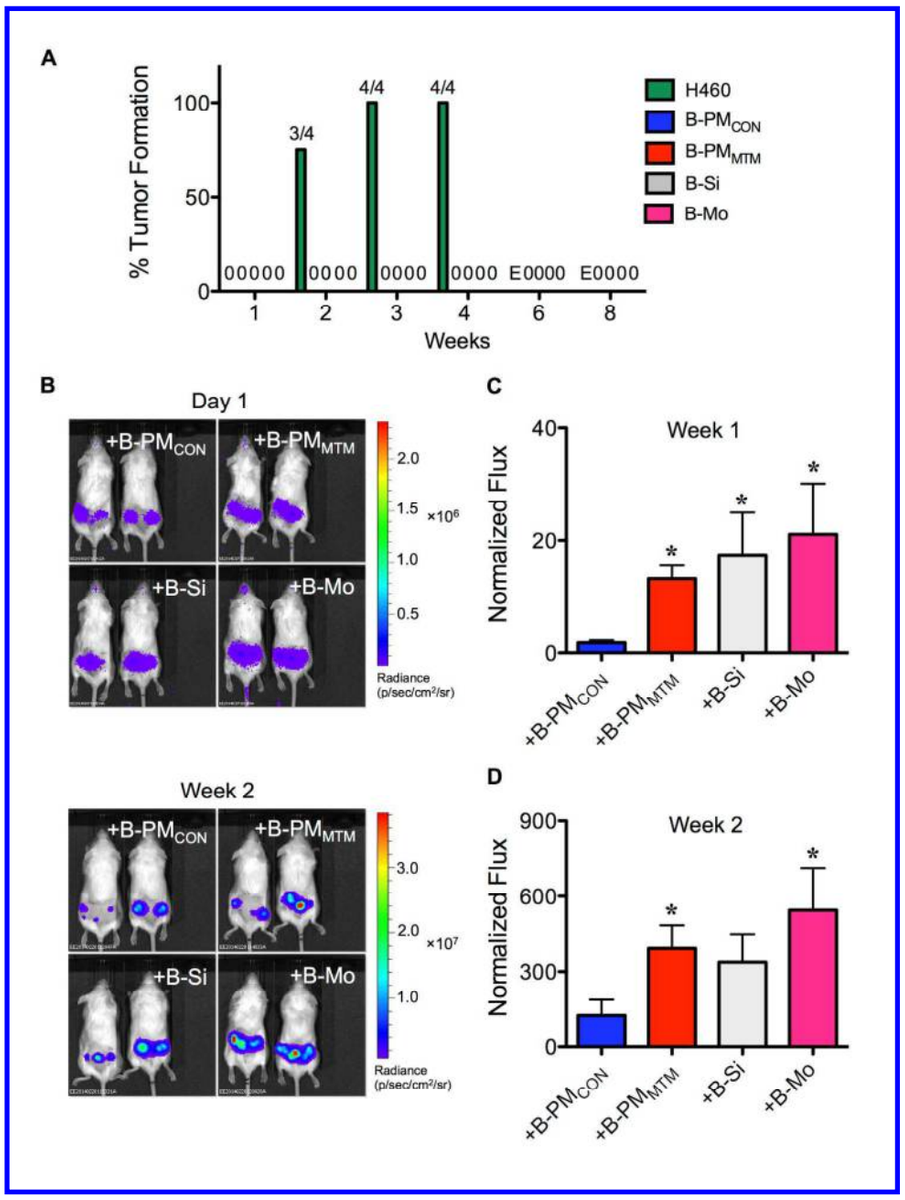


Figure 5. Chronic PM_{MTM} exposure enhances migration of human bronchial epithelial cells. (*A*) Confluent monolayers of B-NTX, B-PM_{CON}, B-PM_{MTM}, B-Si and B-Mo cells were wounded, and the cells were allowed to migrate for 24 hours. Wound space was visualized under a phase contrast microscope and analyzed by comparing the change in wound space as a percentage of wound closure. Data are mean ± SD (n = 3). *$P < 0.05$ (power > 80%) *vs.* passage control B-NTX cells. **$P < 0.05$ (power > 80%) *vs.* B-PM_{CON} cells. (*B*) Representative micrographs from three independent experiments were shown.



526

527 **Figure 6.** Chronic PM_{MTM} exposed cells promote tumor formation of human non-small
528 cell lung cancer H460 cells in mice. (*A*) Growth kinetics of H460 or transformed B-
529 PM_{CON}, B-PM_{MTM}, B-Si and B-Mo cells (1×10^6 cells) when SC injected into the NSG
530 mice alone. E indicates the end of experiment. (*B*) Transformed cells at the dose of 6×10^5
531 cells were co-injected with luciferase-labeled H460 cells at the dose of 3×10^5 cells (2:1
532 ratio) into the left and right flanks of NSG mice. Tumor formation was monitored weekly
533 by IVIS bioluminescence imaging. Representative IVIS images of mice at day 1 and
534 week 2 are shown. (*C*, *D*) Normalization of tumor bioluminescence signals at 1 (*C*) and 2
535 (*D*) weeks post-injection to their initial signal at day 1. Data are mean ± SD ($n = 4$). *$P <$
536 0.05 (power > 60%) *vs.* H460 and B-PM_{CON} co-injection.

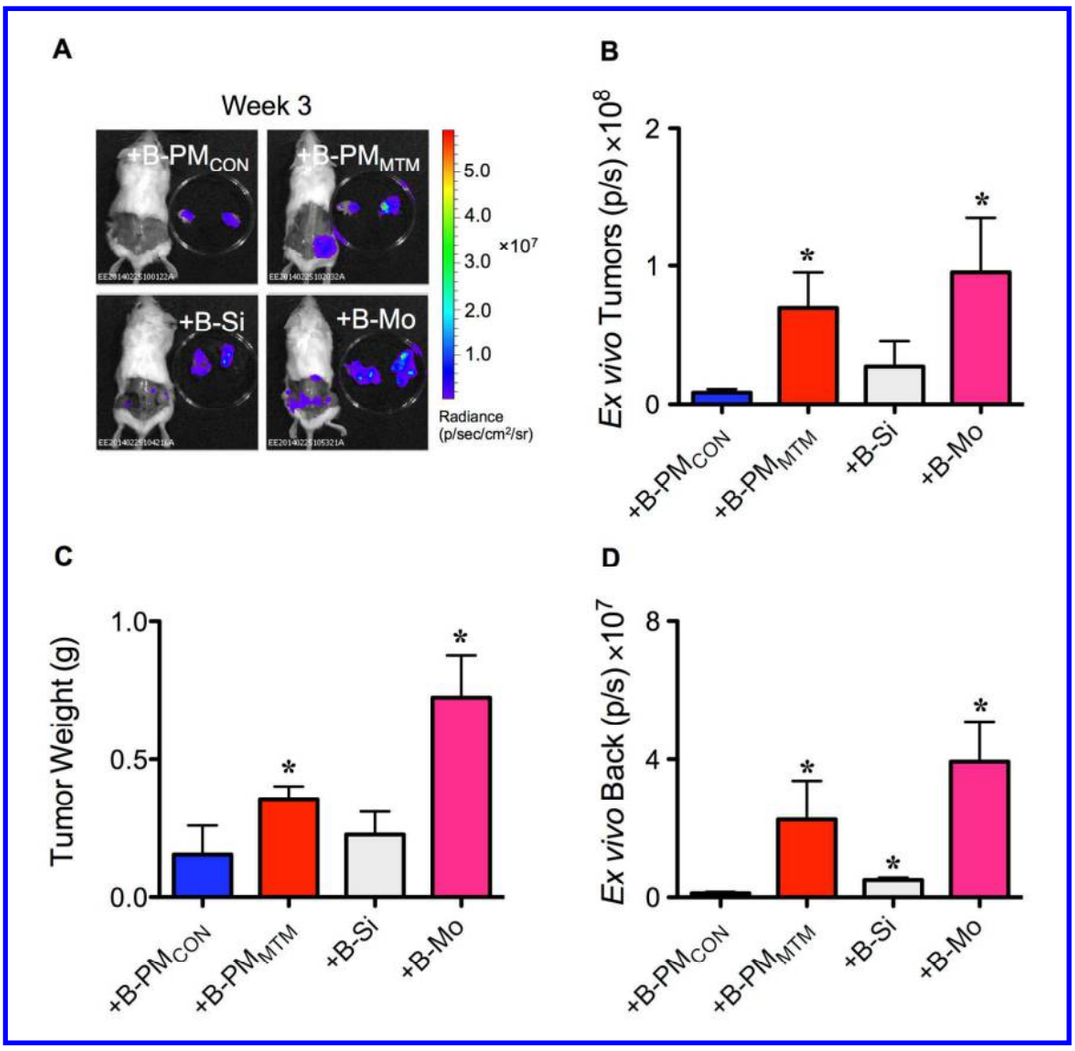


Figure 7. Analysis of *ex vivo* tumors of human lung cancer H460 cells. At 3 weeks post-injection, SC tumors were dissected from mice bearing H460 and B-PM_{CON}, B-PM_{MTM}, B-Si or B-Mo cells. (*A*) Representative bioluminescence images of mice and SC tumors. (*B*) Quantitative analysis of bioluminescence signals from SC tumors. Data are mean ± SD (n = 4). *P < 0.05 (power > 60%) *vs.* H460 and B-PM_{CON} co-injection. (*C*) The weight of dissected SC tumors. (*D*) Quantitative analysis of bioluminescence signals from the back of mice.