Hypomethylation of *Dual Specificity Phosphatase 22* Promoter Correlates With Duration of Service in Firefighters and Is Inducible by Low-Dose Benzo[a]Pyrene

Bin Ouyang, MD, C. Stuart Baxter, PhD, Hung-Ming Lam, PhD, Samrat Yeramaneni, BS, Linda Levin, PhD, Erin Haynes, PhD, and Shuk-mei Ho, PhD

Objective: Firefighters (FFs) are chronically exposed to smoke and products of incomplete combustion, which frequently contain polycyclic aromatic hydrocarbons (PAHs). This study examined the possibility of an association between PAH-induced epigenetic alterations and occupational firefighting exposure. **Methods:** Promoter methylation was analyzed in four genes in blood DNA from 18 FFs and 20 non-FFs (controls). Jurkat and human normal prostate epithelial cells were treated with benzo[a]pyrene to ascertain the epigenetic effects of this type of agent. **Results:** Firefighters had a higher prevalence of *dual specificity phosphatase 22*-promoter hypomethylation in blood DNA ($P = 0.03$) and the extent of hypomethylation correlated with duration of firefighting service ($P = 0.04$) but not with age. Benzo[a]pyrene reduced promoter methylation and increased gene expression of the same gene in Jurkat and normal prostate epithelial cells. **Conclusions:** Cumulative occupational exposure to combustion-derived PAHs during firefighting can cause epigenetic changes in promoters of specific genes.

Learning Objectives

- Become familiar with the potential health risks associated with exposure to polycyclic aromatic hydrocarbons (PAHs) in firefighters and the suggested role of epigenetic changes.
- Discuss the new in vitro evidence on gene hypomethylation in firefighters and the associated exposure factors.
- Review the authors' conclusions regarding PAH as a potential cause of epigenetic changes in firefighters and on the implications for monitoring occupational exposure to toxicants.

Evidence is rapidly emerging that environmental exposure can perturb the epigenome, resulting in potential biomarkers of exposure or "discriminators" of individual susceptibility.[1,2] To our knowledge, epigenetic alteration resulting from exposure from incomplete combustion products, including polycyclic aromatic hydrocarbons (PAHs), has yet to be evaluated. Firefighters (FFs) work in a special environment and are potentially exposed to a wide range of toxic substances both at the fire scene and at the fire station, including toxic gases, ultrafine particulates, and organic compounds, including PAHs, which may be present in the vapor and/or particle-bound states or both.[3–6] These substances may be absorbed via inhalation and ingestion. In addition, FFs at those sites that have relative highest susceptibility to transdermal absorption have skin that is often covered with smoke-derived deposits, suggesting this route is especially significant. Self-contained breathing apparatus may be removed prematurely after initial fire suppression, resulting in unintended inhalation of toxicants.[5] Firefighters are continuously exposed to PAHs from additional sources, including vehicle diesel exhaust and smoke-derived deposits on protective gear, clothes, and fire-suppression equipment in the fire house while on call.[4]

In comparison with nonfirefighting populations, FFs are at risk for multiple types of disease, including coronary heart disease, neurodegenerative disease, pulmonary disorders, and several types of cancer.[4,7,8] It has been proposed that increased risk of these diseases is related to occupational PAH exposure.[3–5]

The chemical class of PAHs includes many carcinogens, atherosclerotic agents, and neurotoxins.[9–12] Benzo[a]pyrene [B(a)P] is the most studied compound in this class, and together with other PAHs, is formed by the incomplete combustion of organic materials. Firefighters are routinely exposed to elevated levels of PAHs during the course of fire suppression. 1-Hydroxypyrene, a PAH metabolite, could be detected in the urine of FFs within 6 to 7 hours and more than 4 days after a fire suppression event despite the use of appropriate protective equipment.[3]

Polycyclic aromatic hydrocarbons and their metabolites are genotoxic[9] as a result of the formation of DNA adducts and oxidative DNA damage. Traditionally, PAH genotoxicity is believed to initiate carcinogenesis, atherogenesis, neurodegeneration, and the induction of inflammation.[10,11] Emerging evidence, though sparse as yet, suggests that PAHs can also directly or indirectly induce epigenetic changes relevant to disease development. The best examples recently reported are global or gene promoter–specific DNA methylation changes. Such alterations were reported in blood cell DNA from coke-oven workers[13] and in umbilical cord blood cell DNA from children born to mothers living in traffic-laden city environments.[14] These epigenetic alterations may therefore reflect a history of exposure to PAHs.

DNA methylation is a fundamental mechanism for epigenetic control of gene expression and the maintenance of genomic integrity through preservation of higher-order chromatin assembly.[15] DNA methylation patterns are established by a tightly regulated intrinsic program beginning before implantation and lasting through pre- and perinatal life.[16] Some cell-/tissue-specific patterns remain flexible and are reprogrammable during susceptible windows of development, as well as throughout later life, in response to environmental

From the Division of Environmental Genetics and Molecular Toxicology (Drs Ouyang, Baxter, Lam, and Ho) and Division of Epidemiology and Biostatistics (Mr Yeramaneni, and Drs Levin and Haynes), Department of Environmental Health, Center for Environmental Genetics, University of Cincinnati Cancer Institute, College of Medicine, University of Cincinnati, and Cincinnati Veterans Affairs Medical Center (Dr Ho), Cincinnati, Ohio.

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Address correspondence to: Shuk-Mei Ho, PhD, Department of Environmental Health, Kettering Complex, Room 128, 3223 Eden Ave, University of Cincinnati Medical Center, Cincinnati, OH 45267 (shuk-mei.ho@uc.edu).

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perturbations[1,16,17] or disease development.[1,18–20] Because of the stable, yet modifiable, nature of DNA methylation, both global and gene-specific changes can serve as biomarkers of exposure to toxicants, pre- and established disease states, or both.[14]

The main objective of this study was to investigate an important but unchartered research area of high relevance to the occupational health of FFs and others (eg, emergency responders to oil fires and wildfires and certain military personnel) exposed to incomplete combustion products. We examined promoter methylation status of four genes: glutathione S-transferase pi-1 (*GSTP1*), interferon-γ (*IFN-*γ), *RAD21 homolog* (*Schizosaccharomyces pombe*), and *dual specificity phosphatase 22* (*DUSP22*) in whole-blood DNA from active professional FFs and control individuals (non-FFs). The promoter methylation status of these genes was previously reported to be correlated with environmental exposures to traffic-related PAHs, diesel exhaust particles, and smoking.[1,14,21,22] Whether the prototype PAH and ubiquitous smoke constituent B(a)P could induce analogous epigenetic changes has also been investigated in vitro. The effect of B(a)P on gene expression, the immediate consequence of altered gene promoter methylation status, was also examined. An increased risk for prostate cancer in FFs has been reported.[23] To demonstrate whether the study cohort was free of preexisting or existing occupation-related prostate abnormalities, prostate specific antigen (PSA) was used as a surrogate marker of prostate health. To verify that the expression of *DUSP22* is regulated by the status of promoter methylation of this gene, cells were treated for assessment of their demethylation susceptibility with 5-aza-2′-deoxycytidine (5-aza-dC), a ubiquitous inhibitor of cytosine methylation on DNA.

MATERIALS AND METHODS

Subjects and Blood Sample Collection

We recruited new and experienced FFs from the City of Cincinnati Fire Service and Radiation Safety officers from the University of Cincinnati. All potential study participants were informed about the study procedures and signed a University of Cincinnati institutional review board consent form. Participants completed a questionnaire containing occupational and medical history and were asked to provide a 5-mL sample of blood. The sample was collected at the beginning of the study into BD Vacutainer ethylenediaminetetraacetic acid tubes (BD Corp, Franklin Lakes, NJ) by a member of the attending paramedic team when FFs returned to the firehouse after a fire event. Blood was transported at 4°C within 1 hour of collection to the laboratory, aliquot, and stored at −80°C.

Cell Lines and Treatment

Immortalized human Jurkat T lymphocytes were purchased from the American Type Culture Collection and maintained in Roswell Park Memorial Institute 1640 medium (Invitrogen, Carlsbad, CA). The immortalized human normal prostate epithelial cells (NPrEC) were established in our laboratory and maintained in Dulbecco Modified Eagle Medium (Invitrogen) and Defined Keratinocyte serum-free medium (Invitrogen) with growth supplement (Invitrogen) as previously described.[24] For B(a)P treatment experiments, cells were incubated with 0.1, 1, or 10 nM of B(a)P, or with dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO) as vehicle control, for up to 2 weeks. The culture medium was replenished with fresh B(a)P or DMSO every 2 days. For 5-aza-dC treatment, cells were exposed to 0.5 or 1 μM 5-aza-dC (Sigma-Aldrich), or DMSO for 5 to 6 days. Cells were dissociated with trypsin and collected for both DNA and RNA extraction at the endpoints indicated.

TABLE 1. Subject Characteristics and Occupational Exposure

Sample	FF $N = 18$	Non-FF $N = 20$
Sex		
Male	17	18
Female	1	2
Age, yrs		
Range	30–53	23–53
Mean	41	34
Race		
White	14	20
Black	4	0
Firefighting service years	9–36	0
Mean	18	0
Cigarette smoking		
Current smoker	2	3
Never smoker	16	17

FF, active professional firefighter; Non-FF, nonfirefighting control.

Methylation-Specific Polymerase Chain Reaction and Bisulfite Sequencing

DNA from whole blood or cultured cells was extracted with DNeasy Blood & Tissue kit (Qiagen, Valencia, CA). A 200 ng of genomic DNA from each blood sample or cell culture was chemically modified with sodium bisulfite using the EZ Methylation Kit (Zymo Research, Irvine, CA). Primers for methylation-specific polymerase chain reaction (MS-PCR) and bisulfite-PCR (BS-PCR) are listed in Supplemental Table 1 at http://links.lww.com/JOM/A91. MS-PCR and BS-PCR were carried out as previous described.[14,25] The PCR primers were designed on the basis of the genomic sequences from GenBank databases at the National Center for Biotechnology Information using the online software MethPrimer (www.urogene.org/methprimer/index1.html). Primers for BS sequencing were designed to amplify a 484 bp (436 to 919) fragment downstream of the transcriptional start site encompassing the predicted CpG island of *DUSP22* (BS-PCR region is indicated in Fig. 1A). Bisulfite-PCR amplicons were gel purified and cloned into the pGEM-T vector (Promega Inc., Madison, WI). Six clones were picked from each sample for sequencing (Macrogen Inc., Rockville, MD). DNA methylation data from sequencing were analyzed using the BiQ Analyzer (Max-Planck-Institute for Informatics, Saarbrücken, Germany).[26] Percentage promoter methylation was calculated by an average of the methylation percentage of 45 CpGs of the six clones in an individual sample.

RNA Isolation and Quantitative Reverse Transcription–PCR

Total RNA isolation was performed as previously described.[27] RNA was reverse transcribed to complementary DNA using SuperScript III First-Strand Synthesis System (Invitrogen). Polymerase chain reaction primers specific for *DUSP22* and the housekeeping gene hypoxanthine phosphoribosyltransferase (HPRT) have been described previously.[14] Quantitative PCR was conducted with 2X SYBR Green Universal PCR Master Mix in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The 2-$\Delta\Delta$Ct method was used to calculate the relative expression levels of a transcript by normalization to the level of HPRT messenger RNA. All reactions were run in triplicate and three independent assays were performed on each sample. Values from vehicle-treated

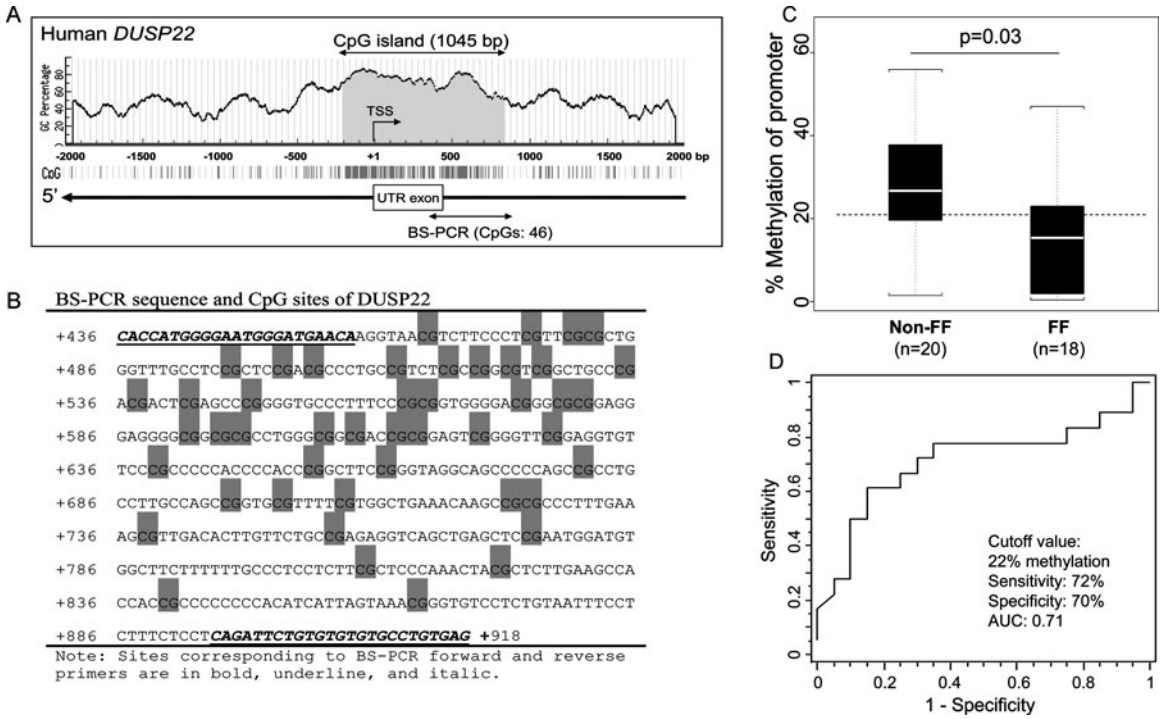

FIGURE 1. Hypomethylation of the *dual specificity phosphatase 22* (*DUSP22*) promoter in professional residential firefighters (FFs). **A,** Schematic diagram of the cytosine-guanine (CG) content in the 5′ flanking region of the *DUSP22* gene. A 1045-bp CpG island is shaded in blue. The transcription start site is marked with a bent arrow. The untranslated exon is marked with a box. The bisulfite–polymerase chain reaction (BS-PCR)–amplified region is indicated by a left–right arrow. **B,** The sequence corresponding to the BS-PCR–amplified region. CpG sites are shaded in green. **C,** DNA from whole blood of active professional FFs and non-FFs (controls) were quantitatively analyzed for percent methylation of *DUSP22* promoter region by BS sequencing. Six clones from each sample were sequenced. Horizontal dashed line indicates the 22% methylation value. **D,** A receiver operating characteristic curve was drawn to graphically assess the discriminating power of the gene promoter methylation for differentiation of samples with or without occupational firefighting exposure.

cells were assigned arbitrarily an abundance value of 1.0, which served as a comparison standard for values from other groups with different doses of agents under the same treatment conditions.

Measurement of PSA

The concentration of blood PSA was measured by sandwich enzyme-linked immunosorbent assay according to manufacturer's protocol (Bioquant, San Diego, CA). Plasma was separated from subjects' whole blood and 50 μl of plasma, control, or PSA standard was added to each well of enzyme-linked immunosorbent assay plate. Each reaction was carried out in duplicate. Samples were independently measured twice. Concentrations of plasma PSA were calculated from a PSA standard curve.

Statistical Analysis

For each worker, summary measures of methylation were estimated from the analysis of indicator variables specifying the presence (1) or absence (0) of methylation at 45 sites and six clones per site. Subject-specific clones (site averages) were analyzed as outcomes using mixed effects regression modeling. Nonparametric Kruskal-Wallis and Mann-Whitney *U* tests were performed to compare central tendencies among worker categories and FFs compared with non-FFs. Worker category means and standard errors were obtained from a model assuming that workers were a representative sample of workers from similar occupations (a random effect). From this model, the variability of between-subject means within each worker category was estimated. Worker means and within-subject clone variability were estimated from a model in which worker identification was modeled as a fixed effect. Clone variation was estimated from the repeated measurements of clone values within each subject. Sensitivities and specificities corresponding to the continuum of methylation percentages were calculated, and a receiver operating characteristic graph was drawn. The optimum cutoff point of percent methylation for discriminating between the two groups was obtained by minimization of the distance from the (1, 1) coordinates of the *x–y* axes to the receiver operating characteristic graph. The analyses were performed using SAS for Windows, version 9.2, (SAS Institute, Cary, NC).

RESULTS

Study Participants

We recruited 38 participants into the study including 18 active professional FFs and 20 controls with no previous professional firefighting experience (non-FFs). Fire fighters had a fire repression service ranging from 9 to 25 years. Non-FFs had never participated in fire suppression. Subject characteristics are listed in Table 1.

DUSP22 Promoter Is Hypomethylated in Active Professional FFs

To investigate the relationship between the extent of promoter methylation and length of occupational firefighting experience, we assessed the promoter methylation status of four human genes: *GSTP1, IFN-γ*, *RAD21*, and *DUSP22* in whole-blood DNA. The promoter regions of *GSTP1*, *IFN-γ,* and *RAD21* showed no significant difference in their methylation statuses between FFs and

non-FFs (see Supplemental Table 2 at http://links.lww.com/JOM/A92). Only *DUSP22* (see Fig. 1A and B for gene organization and location of primers) was found to be significantly hypomethylated in its promoter region in active FFs compared with the control group (non-FFs) (Fig. 1C). The percentage of promoter methylation was calculated as the average percentage methylation of the 45 CpGs across the CpG-rich island of *DUSP22* (see Materials and Methods and Fig. 1). The percentage promoter methylation of *DUSP22* in blood was statistically different between FFs (median, 15%) and non-FFs (median, 27%), with $P = 0.03$ (Fig. 1C). There was extensive hypomethylation (<10% methylation of CpGs in the promoter region), in 7 of 18 FFs(39%), and 2 of 20 non-FFs (10%). A receiver operating characteristic graph drawn (Fig. 1D) to graphically assess the power of gene promoter methylation to discriminate between samples from FFs and non-FFs showed an optimal discrimination at 22% methylation, corresponding to 72% sensitivity (95% confidence interval, 47% to 88%) and 70% specificity (95% confidence interval, 48% to 88%). We have included a Supplemental Table 3 at http://links.lww.com/JOM/A93 to describe the magnitude of two sources of variability of the data, including estimated standard deviations of methylation percentage among workers in each category and estimated standard deviations of clone methylation percentage in each category. The results indicated that firefighting activity is associated with hypomethylation of the *DUSP22* promoter.

Hypomethylation of the *DUSP22*-Promoter Region Is Not Age Related but Is Correlated With Years of Occupational Experience

Firefighters are chronically exposed to a wide range of toxic agents. Accordingly, the effects of some of these agents might be expected to accumulate with years of active service. We found that the methylation status of the *DUSP22* promoter region was significantly correlated with firefighting service years ($r = -0.48$, $P = 0.04$) in FFs (Fig. 2A) but not with age ($r = 0.14$, $P = 0.56$) in non-FFs (Fig. 2B).

All Subjects Have Normal Serum PSA Levels

Firefighters have been reported to have higher prevalence of prostate cancer.[4] The serum PSA test is a first-line assay for early detection of prostatitis, hyperplasia, and cancer in the prostate. To determine if FFs in our cohort had abnormal prostate functions as a result of long-term exposure to smoke-related toxicants, to analyze further the association of epigenetic changes with prostate disease, and to rule out preexisting prostate diseases in controls, we measured blood PSA concentrations of study subjects. They were all within the normal range (data not shown).

5-Aza-dC Induces Promoter Hypomethylation and Increases Transcript Expression of *DUSP22*

Treatment of Jurkat cells and NPrEC with 5-aza-dC for 5 to 6 days resulted in a dose-dependent hypomethylation of the *DUSP22* promoter compared with vehicle treatment (Fig. 3, upper panel). A significant increase in messenger RNA expression of *DUSP22* in 5-aza-dC treated cells was concurrently detected (Fig. 3, lower panel), indicating that the expression of *DUSP22* was regulated by the status of promoter methylation of this gene.

B(a)P Induces Promoter Hypomethylation and Increases Transcript Expression of *DUSP22* In Vitro

We first carried out a pilot experiment to examine the toxicity of B(a)P on cell growth and optimized the concentration required for induction of DNA methylation in NPrEC. We found that B(a)P at concentrations 10 nM or less did not significantly affect cell growth, but concentrations 100 nM or more inhibited cell growth and caused cell death. We further found that hypomethylation of the gene promoter could be induced at B(a)P concentrations 1 nM or more after 1 week and 0.1 nM or more after 2 weeks of treatment. Therefore, 2 weeks of treatment with B(a)P at concentrations of 0.1 to 10 nM was chosen for our study.

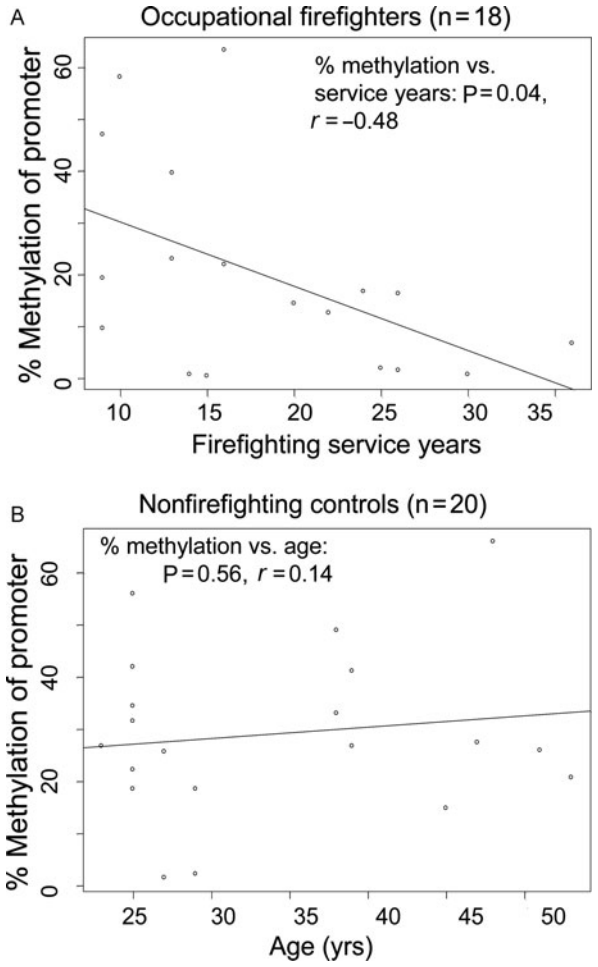

FIGURE 2. Hypomethylation of *dual specificity phosphatase 22* (*DUSP22*) promoter is not age related but correlated with duration of occupation. Spearman correlations were calculated to assess associations in each category. **A,** A total of 18 firefighter veterans were analyzed for correlation of duration of firefighting service with percentage of methylation. **B,** A total of 20 controls without firefighting exposure were analyzed for association of age with methylation percentage.

Treatment of Jurkat cells and NPrEC with B(a)P at low concentrations (0.1 to 10 nM) for 2 weeks resulted in a significant dose-dependent hypomethylation of the *DUSP22* promoter region (Fig. 4, upper panel), and concordant increases in *DUSP22* transcript expression when compared with vehicle treatment in both cell lines (Fig. 4, lower panel). Treatment of Jurkat cells and NPrEC with B(a)P over the same concentration range (0.1 to 10 nM; 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) did not affect cell growth as measured by cell counting and the MTT assay (data not shown). Taken together, these data indicated that B(a)P is not cytotoxic at concentrations that can elicit promoter hypomethylation and increases gene expression in *DUSP22* in Jurkat cells and NPrEC.

DISCUSSION

Although there is evidence that certain PAHs cause cancer and other diseases via genotoxicity,[10,11] we hypothesize that PAHs may contribute to development of these diseases through epigenetic

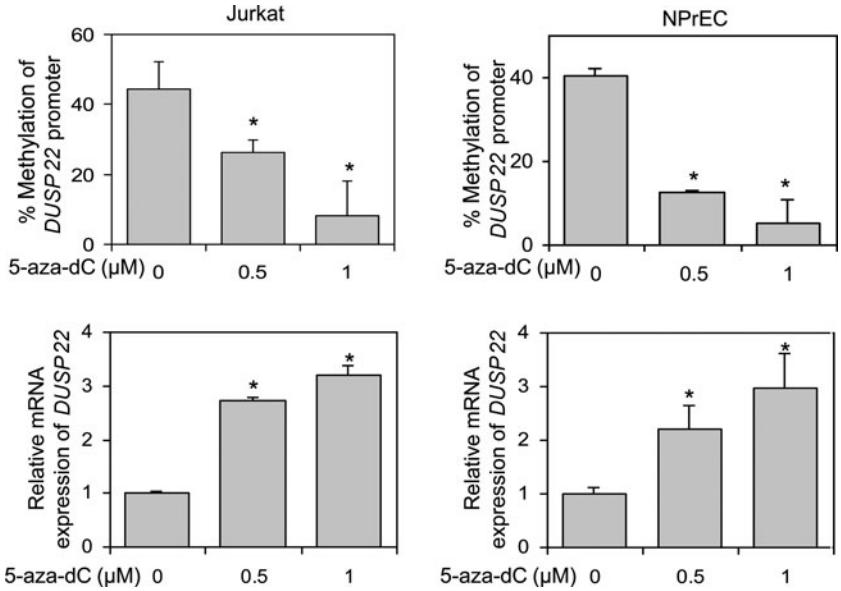

FIGURE 3. 5-Aza-deoxycytosine (5-aza-dC)–induced hypomethylation of *dual specificity phosphatase 22* (*DUSP22*) promoter and transcript expression. Jurkat cells and human normal prostate epithelial cells (NPrECs) were treated with 5-aza-dC for 6 days. DNA and RNA were extracted for determination of the induction of promoter hypomethylation (upper panel) and messenger RNA (mRNA) expression (lower panel). Six clones from each sample were sequenced. *Statistically significant compared with mock treatment 0 μM).

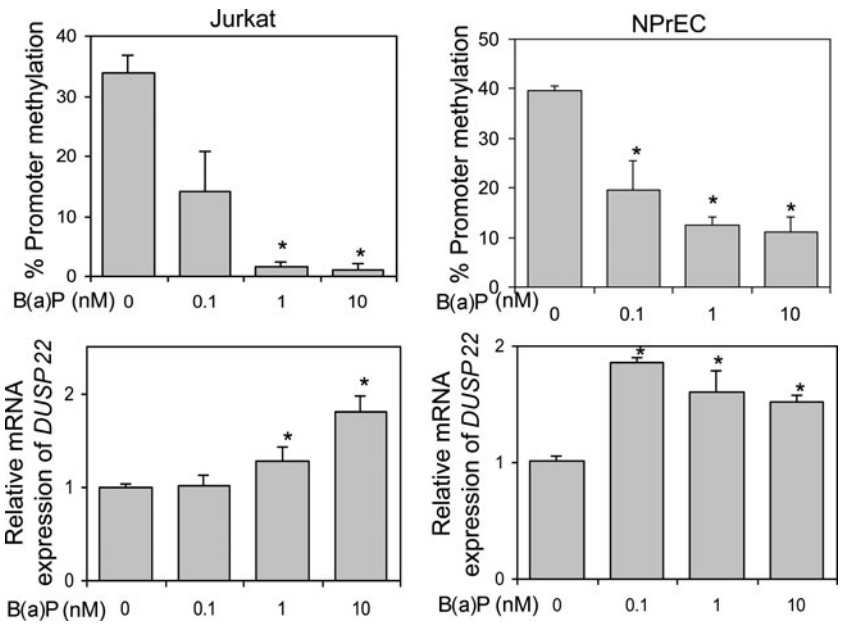

FIGURE 4. Benzo[a]pyrene (B(a)P) induced hypomethylation of *dual specificity phosphatase 22* (*DUSP22*) promoter and degree of transcription. Jurkat cells and human normal prostate epithelial cells (NPrECs) were treated with various concentrations of B(a)P for 2 weeks. DNA and RNA were extracted for determination of the promoter methylation status (upper panel) and messenger RNA expression (lower panel). Six clones from each sample were sequenced. *Statistically significant compared with mock treatment (0 nM).

reprogramming[1] in FFs as a result of long-term occupational exposure. Here we provide the first evidence for this hypothesis. A higher prevalence (39% vs 10%) and degree (median, 15% vs 27%) of *DUSP22* promoter hypomethylation was detected in active professional FFs when compared with a control group (non-FFs). Promoter hypomethylation of the *DUSP22* gene was not found to be age related but correlated with length of occupational service. Of the 7 cases with extensive promoter hypomethylation, 6 had at least 14 years of firefighting experience, and 1 had 9 service years and was also a current smoker. Of the two control individuals with significant *DUSP22* promoter hypomethylation, one was reported to be constantly exposed to second-hand smoking and the other not to have an apparent contributing factor. At this point, because of the low number of smokers in our cohort (2 FF and 3 non-FF, Table 1), it is not possible to conclude whether smoking contributes to epigenetic alteration of the *DUSP22* promoter.

Observational studies have consistently indicated an increased prostate cancer incidence in FFs.[4] Here we showed that exposure of NPrEC to low concentrations of the smoke-derived carcinogen B(a)P induced *DUSP22* promoter hypomethylation and increased gene expression. Since some of the subjects in our cohort are older than 45 years (FFs, 30 to 53 years old; non-FFs, 23 to 53 years old), the possibility existed that some of them might have harbored prostate cancer. It was logical to question if *DUSP22* promoter hypomethylation is a reflection of prostate disease in this cohort. We therefore measured serum PSA levels, a mainstay test for prostate diseases (benign and malignant growth and prostatitis), and blood DNA *GSTP1* promoter hypermethylation, a widely confirmed biomarker of prostate cancer[22,28] in our subjects. Collectively, our data suggested that the observed hypomethylation of *DUSP22* promoter was not a consequence of prostate disease but likely related to exposure to smoke or products of incomplete combustion.

One limitation of this study was that we did not examine biomarkers of other diseases associated with firefighting. In this regard, it will be of significant interest to measure biomarkers associated with atherosclerosis-related cardiovascular disease and

oxidative stress–related disorders. These may include homocysteine, C-reactive protein, and other cardiovascular/atherosclerosis biomarkers of clinical values in our future studies. Applying the same reasoning, our impending studies will include blood markers of gonadal function (serum levels of testosterone, luteinizing hormone, anti-Müllerian hormone, and inhibin-B) and other malignancies such as ovarian (CA 125[29]) and testicular (α-fetoprotein[30]) cancers. Such an approach is expected to generate new insights into the health of FFs because PAHs and other smoke-derived toxicants are known to have adverse impacts on fertility and reproductive function,[31] which could be major health concerns for younger FFs.

In a previous study,[14] we identified RAD21 and *DUSP22* as genes whose promoter methylation statuses were linked to transplacental PAH exposure using umbilical cord blood DNA from a cohort of children born to mothers living in a traffic-laden city neighborhood. Asthma incidence (~30%) in this cohort is among the highest in the nation. Recently, we reported that *IFN-*γ also exhibits a similar relationship with maternal PAH exposure in the same cohort of children (Tang YW, unpublished data). Interestingly, among these three genes, we found only the promoter of *DUSP22*, not *RAD21* or *IFN-*γ, to be modified by firefighting activity. This result was in contrast to findings in our children's study and raised the intriguing question of why firefighting triggered promoter hypomethylation in *DUPS22* but not in the other two genes. Our in vitro experiments clearly demonstrated that low concentrations of B(a)P (0.1 to 10 nM) induced *DUSP22* promoter hypomethylation and increased gene expression. The *IFN-*γ promoter has also been shown to be sensitive to low concentrations of B(a)P and undergo promoter hypermethylation and gene silencing in vitro in our laboratory (Tang YW, unpublished data) and in Bagg Albino mice inhaling diesel exhaust particles in other laboratories.[21] An overall rational interpretation of these findings is therefore that epigenetic alteration of gene-specific promoters is highly dependent on the context of the exposure. Polycyclic aromatic hydrocarbon exposure in the children's study was traffic related and transplacental, whereas, in the current study of FFs, exposure was in the form of a mixture of smoke-derived toxicants, likely absorbed via inhalation, ingestion, or by transdermal absorption.

The *DUSP22* gene encodes an enzyme that belongs to a family of atypical dual specificity phosphatases.[32] The long 3.0-kb transcript of *DUSP22* has a wide tissue distribution but the shorter 1.3-kb variant has restricted expression in the testes and liver.[33] The enzyme is an upstream regulator of several mitogen-activated protein kinase pathways but its function is highly dependent on cell type and context. Ectopic expression of *DUSP22* in COS7 cells preferentially dephosphorylates p38 and Jun amino-terminal kinase, but not extracellular signal–regulated kinase,[34] whereas it dephosphorylates extracellular signal–regulated kinase in Jurkat cells.[35] Mouse embryonic stem cell experiments clearly demonstrate the central role of *DUSP22* in cytokine-induced Jun amino-terminal kinase activation.[33] The enzyme is also involved in estrogen action because it can dephosphorylate the estrogen receptor-α and negate estrogen-induced interleukin 6/signal transducer and activator of transcription 3 signaling.[36] A recent study[37] reported a novel chromosome translocation [t(6;7)(p25.3;q32.3)] that leads to suppression of *DUSP22* in approximately half of anaplastic large cell lymphomas, a type of non-Hodgkin lymphoma, a cancer type that has been reported to be elevated in FFs.[4] On the basis of these reports, *DUSP22* seems to play a key role in inflammatory and proliferative disorders. Findings from our study have now placed *DUSP22* as one of the few genes whose expression is modified epigenetically in response to environmental exposure. In this regard, we showed that *DUSP22* expression is upregulated upon B(a)P-induced hypomethylation of its promoter in vitro and that *DUSP22* promoter hypomethylation in peripheral blood DNA is associated with firefighting and the duration of active service. It will be of interest for future studies to investigate whether promoter hypomethylation of *DUSP22,* in addition to being a biomarker of toxic exposure to PAH, also has the capability to predict later-life diseases (such as prostate cancer) resulting from long-term exposure to smoke- or other incomplete combustion–derived toxicants (such as PAHs). Findings from such investigations will undoubtedly deepen our understanding of the role played by epigenetics in exposure biology in settings beyond firefighting, particularly those that also involve exposure to products of incomplete combustion of organic materials, such as in situ burning of oil spills, both during peacetime and as a result of military operations (eg, Gulf War I).

This proof-of-concept study indicates that epigenetic changes occur in professional firefighting populations. These modifications may be toxicant-specific, exposure context–specific, or both and result from exposure to complex mixtures of toxic substances emitted from burning and overheated materials. It is therefore essential to identify a panel of epigenetic biomarkers that are linked to the most common toxicant exposures or exposure contexts encountered by FFs and that could estimate the risk of later-life diseases in this and other exposed populations. Such biomarkers may aid in the development of new strategies/technologies for exposure surveillance and disease prevention in the future.

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