Hazard evaluation of air pollution by using the key characteristics approach

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Abstract. The key characteristic (KC) as a new approach based on mechanistic evidence has been shown by the IARC Monographs Program working group. Human carcinogens like exhibit one or more key characteristics that are related to how they cause cancer, and different carcinogenic agents exhibit different spectra of these key characteristics. Air pollution is one of the major concerns of human health for the people who lives in Asia because air pollution contains many different human carcinogens including heavy metals, volatile compounds, electrophile compounds and polyaromatic hydrocarbon compounds. In this study, we investigated air pollution mixtures derived from diesel and gasoline engine exhausts by a mechanism-based approach. We first conducted a KC analysis of organic compounds of diesel exhaust in IARC monograph 105 using data of biological test results from the PubChem database. As a result, it was found that some PAH among those compounds is highly responsive to aryl hydrocarbon receptor, nuclear factor erythroid 2 like 2, NR1I2/3 nuclear receptor subfamily 1 group 1 member 2/3. Further, we analyzed KCs of water-extract mixtures from diesel exhaust particle (DEP-WM) using gene expression values in the mouse lung responded induced by exposures to DEP-WM. The KC of DEP-WM showed lipid related-nuclear receptor signaling and apoptotic pathway, suggesting that DEP-WM affects lipid metabolism in lung tissues. Thus, the KC method will be useful for high-precision assessment of a mixture of air pollution.

1. Introduction

A long-standing task in toxicology and chemical risk management is to set a standard for real life orientated mixtures. Several approaches for chemical mixtures have been discussed to classify the biological characteristics of the substance, estimate the hazard mechanisms and incorporate them into the hazard assessment [1, 2]. The key characteristic (KC) as a new approach based on mechanistic evidence has been shown by the IARC Monographs Program working group [2]. Smith et al described the use of the 10 key characteristics, which are the biological activities of a compound to 1) act as an electrophile either directly or after metabolic activation; 2) be genotoxic; 3) alter DNA repair or cause genomic instability; 4) induce epigenetic alterations; 5) induce oxidative stress; 6) induce chronic inflammation; 7) be immunosuppressive; 8) modulate receptor-mediated effects; 9) cause immortalization; and 10) alter cell proliferation, cell death, or nutrient supply [3]. Human carcinogens like exhibit one or more key characteristics that are related to how they cause cancer, and different carcinogenic agents exhibit different spectra of these key characteristics [3, 4]. Air pollution is one of...
the major concerns of human health for the people who lives in Asia because air pollution contains many different human carcinogens including heavy metals, volatile compounds, electrophile compounds and polyaromatic hydrocarbon compounds [5, 6]. In this study, we investigated air pollution mixtures derived from diesel and gasoline engine exhausts by a mechanism-based approach.

2. Materials and methods

2.1. Using the key characteristics to Identify and summarize mechanistic Information
Analysis was performed in the same manner as described in the paper of Smith et al [3]. The key Characteristics approaches have three steps: Step1) Identifying the relevant information, Step 2) screening and organizing the results, Step 3) Using the Key Characteristics to synthesize mechanistic information and to develop adverse-outcome networks. In this study, chemical lists were taken from tables in WHO IARC 105 monograph [6] as the step 1. “Biological Test Results” in the database of PubChem compounds were collected in the step 2 and then molecular targets in Tox21 bioassay were selected for chemicals of air pollution. Descriptions of the target names are provided in the supplement table.

2.2. Analyze the key characteristics using gene expression
The water extractable fraction of DE particle (DEP-WM) was used for intranasal administration to animals. Gene expression in the mouse lung tissue induced by the DEP-WM exposure was obtained as described in previous study [7]. Briefly, eight-week-old male BALB/c mice were administered by DEP (50 µg/50 µl/mouse) intranasally at once a week for 4 weeks. Then, mRNA expressions were examined using microarray (Agilent). Pathways of altered gene expressions were informed from KEGG and WikiPathways.
3. Results and Discussion

3.1. KC classification of biological activity of aromatic compounds

Previous studies have proposed a pathway for AhR-mediated carcinogenesis by the DE exposure. They estimate the following pathway: when the polycyclic aromatic compound is metabolically activated and binds to AhR, it binds to the DNA upstream region of the target molecule after nuclear translocation and exhibits transcriptional activity [10]. After the transcriptional activity, the hazard activities are caused by abnormal expression or reduction of a group of molecules related to inflammation or cell cycle and proliferation that lead to develop cancer. Before analyzing compounds of diesel exhaust (DE), we applied KC10 to the AhR pathways that Abbas et al proposed (Figure 1), showing the mechanism applied to KC [10].

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**Figure 1.** Organic Extracts from Air pollution PMs exhibits Multiple Key Characteristics Modified from Abbas et al., Environmental Research 2019 [10].

To compare the proposed PM mixture-AHR pathway with biological pathways of individual PAHs within DE, we selected 33 compounds in diesel exhaust (DE) DE that were 1-Nitropyrene, 2-Nitrofluorene, 9-Nitroanthracene, acenaphthene, acenaphthylene, acetaldehyde, acrolein, aniline, anthracene, benzo(a)anthracene, benzene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(e)pyrene, benzo(ghi)perylene, benzo(k)fluoranthene, biphenyl, chrysene, ethylbenzene, fluoranthene, fluorene, formaldehyde, hexane, methanol, methyl ethyl ketone, naphthalene, ortho-xylene (o-xylene), phenanthrene, phenol, propionaldehyde, pyrene, styrene, and toluene that were contained in the reduction in emission levels by new technology engines for various classes of compounds, for the California Air Resources Board Toxic Air contaminants, and for PAHs and nitro-PAHs [8]. Those 33 chemicals input PubChem search to get biological test results. Then activity and target-name in the biological test results were collected. Table 1 shows the summarized 12 organic compound characteristics out of 33 chemicals. Most of the KC was KC8 receptor mediated effect that relates to transcription and signal transduction, except for genotoxicity. They responded to many different nuclear receptors such as AHR, NR1H4, NR1I2, NR1I3, NR3C1, PPARD, PPARG, RARA and THRB.
Table 1. KC classification of biological activity of aromatic compounds selected from technical engines.

| Chemical name | KC1 Electro-philicity, KC2 Genotoxicity | KC3 Altered DNA repair, Genomic Instability | KC4 Epigenetic changes | KC5 Oxidative stress, tumor-promoting | KC6 Inflammation | KC7 Immunosuppression | KC8 Receptor mediated effects | KC9 Immortalization, Cell proliferation, Inhibit apoptosis, Nutrient supply | KC10 Cell growth, cell proliferation |
|----------------|----------------------------------------|--------------------------------------------|-----------------------|----------------------------------------|-----------------|----------------------|-----------------------------|-----------------------------|-----------------------------|
| 1-Nitropyrene  | positive                                | NFE2L2                                    |                       | antiAR                                 | NR1I2, NR3C1, PGR |                      |                             | ATAD5                        |                             |
| 2-Nitrofluorene| positive                                | NFE2L2                                    |                       | antiAR                                 | ESR1, AHR       |                      |                             |                             |                             |
| 9-Nitroanthracene| positive                             | H2AX, CYP19A1                              |                       | antiAR                                 | NR1I2, NR1I3, PGR |                      |                             |                             |                             |
| Acetaldehyde  | positive                                |                                          |                       | antiAR                                 | antiESR1, GLI3, JUN |                      |                             | C ASP7, TRPA1                |                             |
| Benz(a)anthracene | positive                              | HDAC9, NFE2L2, NFKB1                     |                       | antiAR                                 | AHR, BP3        |                      |                             | ATAD5, TP53                  |                             |
| Benzo(a)pyrene | positive                                | HIF1A, NFE2L2                             |                       | antiAR                                 | AHR, NR1H4      |                      |                             | CASP7, TP53, VDR             |                             |
| Compound Type       | Compounds                        | Gene Expression                                                                 |
|--------------------|----------------------------------|--------------------------------------------------------------------------------|
| Benzo(b)fluoranthene | Positive                        | HIF1A, NFE2L2, HSPB1, JUN, TP53, VDR, SMAD2                                     |
| Benzo(e)pyrene     | Positive                        | HIF1A, NFE2L2, NFKB1, AHR, AR, SMAD2, SMAD3, TP53                              |
| Benzo(ghi)perylene | Positive                        | AR, NFE2L2, TP53, NFKB1, NR3C1, PGR, THRB                                      |
| Benzo(k)fluoranthene | Positive                     | NFE2L2, HSPB1, AHR, antiAR, ESR1, NR1H4, NR1I2, NR1I3, NR3C1, RXRA, CASP7, TP53 |
| Chrysene           | Positive                        | HDAC9, NFE2L2, AHR, AR, ESRRα, ATAD5                                           |
| Pyrene             | Positive                        |                                                                                  |

The blank in KCs indicates negative responsiveness. Acenaphthene, aniline, fluorene, methyl ethyl ketone, propionaldehyde, Styrene and Toluene out of 33 compounds were inactive except genotoxicity in any kinds of bioassay reported in PubChem database.

### 3.2. Analyze the key characteristics using gene expression

DEP used in this animal experiment contained n-alkenes (C15-C35, 56 μg/m³ reference value), pyrene (10.5 μg/m³ reference value), chrysene (5.58 μg/m³ reference value), benzo[e]pyrene (2.33 μg/m³ reference value) and benzo[a]pyrene (1.8 μg/m³ reference value). Gene expression profiles using the microarray bioassay in the mouse lung tissue induced by the DE exposure was obtained as described in previous study [7]. In the gene network pathways based on the gene expression profile of the microarray analysis, adipogenesis, apoptosis, and nuclear receptor and PPAR were significantly detected at $P < 0.05$, but AhR was not influenced, suggesting that exposure to DEP altered genes related to lipid metabolism, such as transcription factors and cell death, and that the transcription activity associated with PPARG might be more affected than AhR. In fact, as shown in Fig. 1, several nuclear receptors and transcription factors were significantly altered.
factors other than AhR are positive in the bioassay data of PubChem for organic compounds from technology engines.

Figure 2. Schematic pathways of adipogenesis and its transcriptional regulation influenced by DE. The bold genes indicate genes altered by the DE exposure. The figures shown in the figure are the following articles: 1. Tontonoz P et al. Mol Cell Biol 1995; 2. Hotamisligil GS Nature 2006; 3. Lowe CE et al. J Cell Sci, 2011; 4. Dobrian AD Front Endocrinol (Lausanne), 2012; 5. Miranda M et al. Am J Physiol Endocrinol Metab 2010; 6. Beale EG, Forest C and Hammer RE Biochimie, 2003; 7. Rosell M, Jones MC and Parker MG Biochim Biophys Acta 2011; 8. Siersbæk R, Nielsen R and Mandrup S. Trends Endocrinol Metab, 2012. This pathway diagram was taken from WikiPathways (www.wikipathways.org/).

There is still a lack of knowledge about the specific chemical fraction within DE or other PMs, which could be mainly responsible of its adverse effects on human health. Therefore, this work aimed to better understand the toxicological effects of organic compounds within PM2.5 from diesel or other technology engine exhaust on human health. No details were given in this article on exposure levels in bioassay. However, DEPs in the recent reports by Borgie et al. [11], Massoud et al. [12], Saliba et al. [13] were 41 – 20 µg/m³, which was comparable to the WHO levels guideline (25 µg/m³) respectively. Mean total concentration of PAH found 9.7 ng/m³ in these particles. In Asia countries, the report indicated that the heating season of 72.6 ng/m³ was higher than the non-heating season concentration of 4.77 ng/m³ [15]. Sun et al reported that the total PAHs (∑16PAHs) varied from 2.82 ± 0.25 to 19.43 ± 2.55 µg/m³ in emission dust samples [16]. Li Y et al reported that PM2.5 concentrations in Beijing, Jinan, and Shanghai were 125.7 µg/m³, 115.9 µg/m³, and 85.1 µg/m³, respectively, and the PAH concentrations in PM2.5 were ranged from 23.2 to 819.8 ng/m³, 25.7 to 727.1 ng/m³, and 8.5 to 133.9
ng/m³, respectively [17]. The values for the health risk assessment estimated by the benzo[a]pyrene equivalent concentration in Beijing and Jinan were 2.39 × 10⁻⁶, 2.57 × 10⁻⁶ and 5.05 × 10⁻⁶, respectively. The two exceeding the 1 × 10⁻⁶ limit (USEPA) were considered likely to pose an inhalation cancer risk to people. Benzo[b]fluoranthene (B[b]F), the most abundant congener, generally reflects the contribution of diesel power generator and diesel trucks and buses. Chrysene (CHR), the second most abundant congener, is often generated during oil distillation process or waste incineration [11]. As shown in Fig. 1, it was revealed that the biological responsiveness of B[b]F and CHR responds to various types of nuclear receptors, similarly to Benzo(a)pyrene. Profiles of gene expression in mouse lung provide multiple nuclear receptor responsiveness of organic compounds derived from DEP. Those data suggest that we have to pay attention multiple organic compounds and their multiple biological responsiveness. It was reported that PM2.5 in the atmospheric environment of China causes inflammation and oxidative stress in macrophages via separate pathways [19]. Argumentation has been that hazard assessment of low-dose mixtures requires multiple indicators [20]. Therefore, evidence-based KC grouping and weighting support appropriate risk assessment of air pollution or organic compounds. It would be essential to develop standard lists of carcinogens and non-carcinogens to support assay qualification and validation in air pollution.

4. Conclusion
In this study, we investigated air pollution mixtures derived from diesel and gasoline engine exhausts by a mechanism-based approach using the public database for chemical and biological/toxicological information. The KC analysis of organic compounds within the diesel exhaust found that several PAHs among those compounds is highly responsive to multiple nuclear receptors. KCs of gene expression profiles in lung tissues of mice exposed to water extracts of DEP indicated lipid metabolism, the apoptotic pathway, and nuclear receptor signals. Thus, the KC method will be useful for high-precision assessment of a mixture of air pollution.

Table 2. Supplemental Table.

| Target name | Assay ID name |
|-------------|---------------|
| AR          | AR - androgen receptor (human) |
| AHR         | AHR - aryl hydrocarbon receptor (human) |
| ARE         | Antioxidant response element (ARE) signaling pathway |
| ATAD5       | ATAD5 - ATPase family AAA domain containing 5 (human) |
| anti-AR     | anti-AR - androgen receptor (human) |
| ESR1        | ESR1 - estrogen receptor 1 (human) |
| anti-ESR1   | anti-ESR1 - estrogen receptor 1 (human) |
| ESR2        | ESR2 - estrogen receptor 2 (human) |
ESRRA - estrogen related receptor alpha (human)

ESRRA with PPARg - estrogen related receptor alpha (human) and PPAR gamma

anti-ESRRA - estrogen related receptor alpha (human)

CASP3/7 - caspase 3/7 (human)

CYP19A1 - cytochrome P450 family 19 subfamily A member 1 (human)

GLI3 - GLI family zinc finger 3 (human)

HDAC9 - histone deacetylase 9 (human)

HIF1A - hypoxia inducible factor 1 subunit alpha (human)

HSPB1 - heat shock protein family B (small) member 1 (human)

JUN - Jun proto-oncogene, AP-1 transcription factor subunit (human)

H2AX - histone H2AX (Chinese hamster)

NFE2L2 - nuclear factor, erythroid 2 like 2 (human)

NFkB1 - nuclear factor kappa B subunit 1 (human)

NR1H4 - nuclear receptor subfamily 1 group H member 4 (human)

NR1I2 - nuclear receptor subfamily 1 group I member 2 (human)

NR1I3 - nuclear receptor subfamily 1 group I member 3 (human)

NR3C1 - nuclear receptor subfamily 3 group C member 1 (human)

PGR - progesterone receptor (human)

PPARA - peroxisome proliferator activated receptor alpha (human)
PPARD - peroxisome proliferator activated receptor delta (human)

PPARG - peroxisome proliferator activated receptor gamma (human)

RARA - retinoic acid receptor alpha (human)

RXRA - retinoid X receptor alpha (human)

SMAD2 - SMAD family member 2 (human)

SMAD3 - SMAD family member 3 (human)

THRB - thyroid hormone receptor beta (human)

TP53 - tumor protein p53 (human)

TRHR - thyrotropin releasing hormone receptor (human)

TSHR - thyroid stimulating hormone receptor (human)

TRPA1 - transient receptor potential cation channel subfamily A member 1 (human)

VDR - vitamin D receptor (human)

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