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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 I 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].

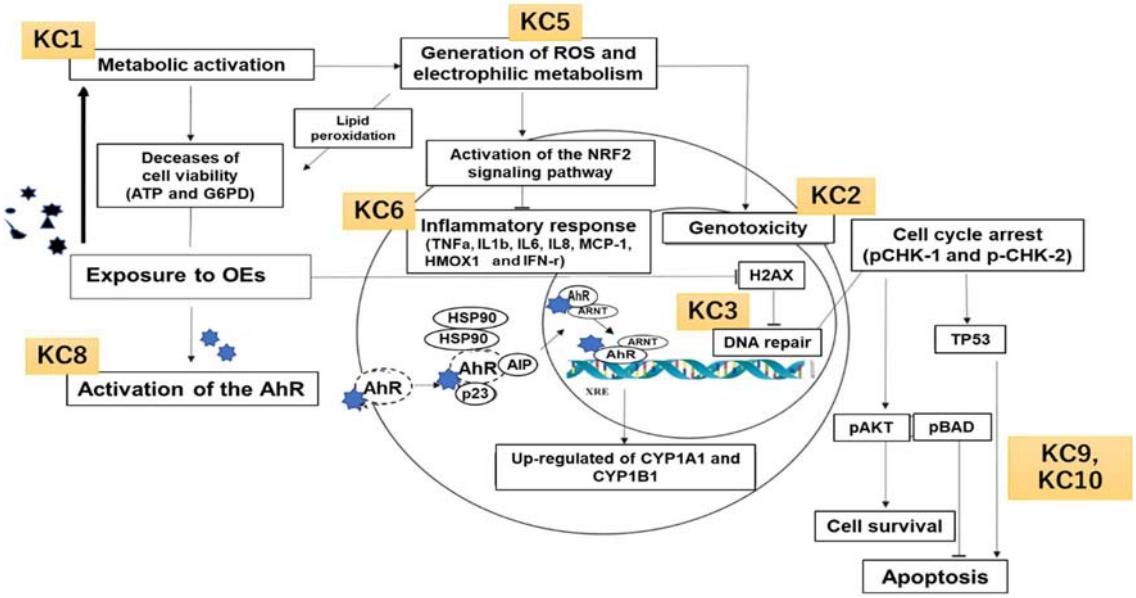

Figure 1. Organic Extracts from Air pollution PMs exhibits Multiple Key Characteristics Modified from Abbas et al., Environmental Research 2019 [10].

To compare the proposed PM mixture-AHR pathway with biological pathways of individual PAHs within DE, we selected 33compounds 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	DNA damage, Mutations KC1 Electro-philicity, KC2 Genotoxicity	Epigenetic changes KC3 Altered DNA repair, Genomic Instability KC4 Epigenetic changes	Oxidative stress, tumor-promoting KC 5 Oxidative stress KC 6 Inflammation	Immunosuppression KC 7 Immuno-suppression	Transcription, signal transduction KC 8 Receptor mediated effects	Cell growth, cell proliferation KC 9 Immortalization KC 10 Cell proliferation, Inhibit apoptosis, Nutrient supply
1-Nitropyrene	positive		NFE2L2		antiAR ESR1 NR1I2 NR1I3 NR3C1 PGR	ATAD5
2-Nitro-fluorene	positive		NFE2L2		antiAR ESR1 AHR NR1I2 NR1I3 PGR	
9-Nitro-anthracene	positive				antiAR ESR1 AHR GLI3 JUN NR1I2 NR1I3 PGR PPARD PPARG RARA	
Acetaldehyde	positive	H2AX	CYP19A1		antiAR antiESR1 antiESRRA GLI3 NR1I2 NR1I3 RARA TRHR TSHR	C ASP7 TRPA1
Benz(a)anthracene	positive	HDAC9	NFE2L2 NFKB1		AHR AR antiAR ESR1 ESRRA ESRRA GLI3 NR1I2 NR1I3 PPARG PGR RARA	ATAD5 TP53
Benzo(a)pyrene	positive		HIF1A NFE2L2	HSPB1	AHR NR1H4 NR1I2 NR1I3 NR3C1 PPARD	CASP7 TP53 VDR

					PPARG RARA	
Benzo(b)fluoranthene	positive		HIF1A NFE2L2 NFKB1	HSPB1	AHR NR1H4 NR1I2 NR1I3 NR3C1 PGR PPARD PPARG RXRA THRB	JUN TP53 VDR SMAD2
Benzo(e)pyrene	positive		HIF1A NFE2L2 NFKB1		AHR AR NR1I3 NR3C1 PPARG RXRA	SMAD2 SMAD3 TP53
Benzo(ghi)perylene	positive				AR NFE2L2 NFKB1 NR3C1 PGR THRB	TP53
Benzo(k)fluoranthene	positive		NFE2L2 NFKB1	HSPB1	AHR antiAR antiESR1 NR1H4 NR1I2 NR1I3 NR3C1 RXRA THRB	CASP7 TP53
Chrysene	positive	HDAC9	NFE2L2		AHR AR ESRRA NR1I2 RARA	ATAD5
Pyrene	positive				AR ESR1 antiESRRA NR1I2 NR1I3 PGR TSHR	

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/m^3 reference value), pyrene (10.5 μg/m^3 reference value), chrysene (5.58 μg/m^3 reference value), benzo[e]pyrene (2.33 μg/m^3 reference value) and benzo[a]pyrene (1.8 μg/m^3 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 other than AhR are positive in the bioassay data of PubChem for organic compounds from technology engines.

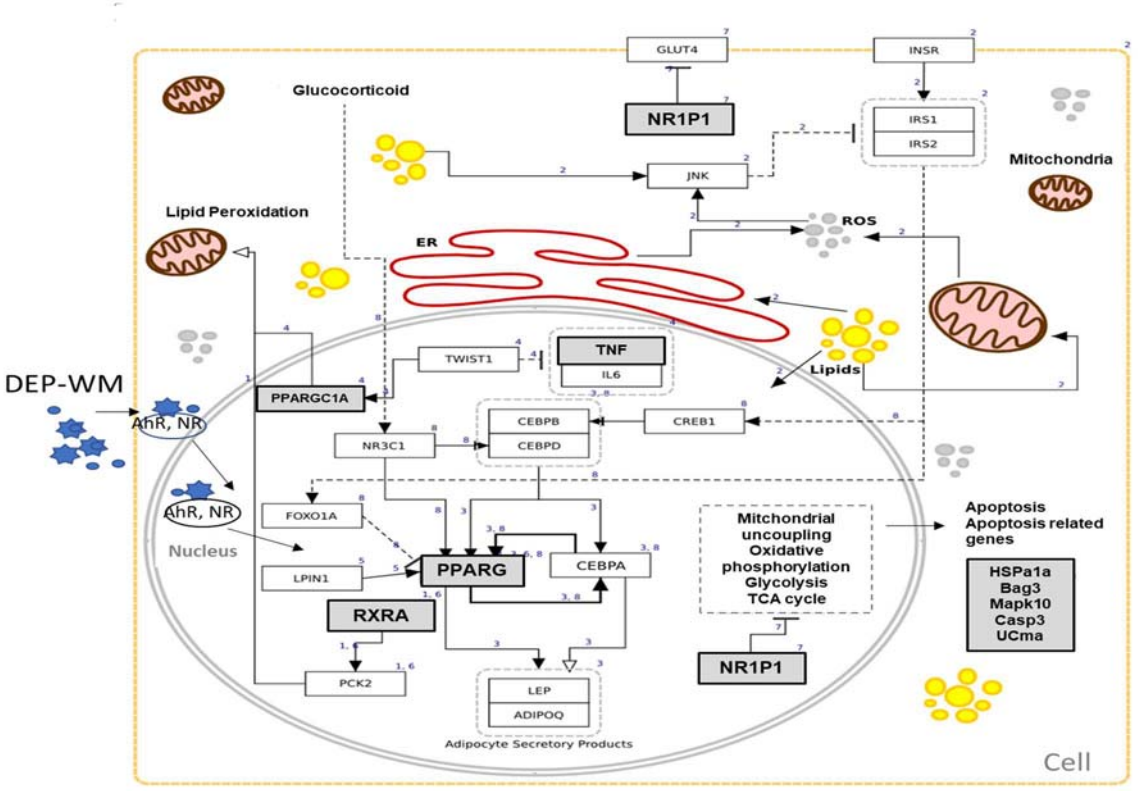

Figure 2. Schematic pathways of adipogenesis and its transcriptional regulation influenced by DE. The bold genes indicate genes altered by the DE exposure. The figures shown in the figure are the following articles:1. Tontonoz P *et al. Mol Cell Biol* 1995; 2. Hotamisligil GS *Nature* 2006; 3. Lowe CE 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/m^3, which was comparable to the WHO levels guideline (25 μg/m^3) respectively. Mean total concentration of PAH found 9.7 ng/m^3 in these particles. In Asia countries, the report indicated that the heating season of 72.6 ng/m^3 was higher than the non-heating season concentration of 4.77 ng/m^3 [15]. Sun et al reported that the total PAHs (∑16PAHs) varied from 2.82 ± 0.25 to 19.43 ± 2.55 μg/m^3 in emission dust samples [16]. Li Y et al reported that PM2.5 concentrations in Beijing, Jinan, and Shanghai were 125.7 μg/m^3 , 115.9 μg/m^3, and 85.1 μg/m^3, respectively, and the PAH concentrations in PM2.5 were ranged from 23.2 to 819.8 ng/m^3, 25.7 to 727.1 ng/m^3, and 8.5 to 133.9

ng/m^3, 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	ESRRA - estrogen related receptor alpha (human)
ESRRA with PPARg	ESRRA with PPARganmma - estrogen related receptor alpha (human)
anti-ESRRA	anti-ESRRA - estrogen related receptor alpha (human)
CASP3/7	CASP7 - caspase 3/7 (human)
CYP19A1	CYP19A1 - cytochrome P450 family 19 subfamily A member 1 (human)
GLI3	GLI3 - GLI family zinc finger 3 (human)
HDAC9	HDAC9 - histone deacetylase 9 (human)
HIF1A	HIF1A - hypoxia inducible factor 1 subunit alpha (human)
HSPB1	HSPB1 - heat shock protein family B (small) member 1 (human)
JUN	JUN - Jun proto-oncogene, AP-1 transcription factor subunit (human)
H2AX	LOC100757539 - histone H2AX (Chinese hamster)
NFE2L2	NFE2L2 - nuclear factor, erythroid 2 like 2 (human)
NFKB1	NFKB1 - nuclear factor kappa B subunit 1 (human)
NR1H4	NR1H4 - nuclear receptor subfamily 1 group H member 4 (human)
NR1I2	NR1I2 - nuclear receptor subfamily 1 group I member 2 (human)
NR1I3	NR1I3 - nuclear receptor subfamily 1 group I member 3 (human)
NR3C1	NR3C1 - nuclear receptor subfamily 3 group C member 1 (human)
PGR	PGR - progesterone receptor (human)
PPARA	PPARA - peroxisome proliferator activated receptor alpha (human)

PPARD	PPARD - peroxisome proliferator activated receptor delta (human)
PPARG	PPARG - peroxisome proliferator activated receptor gamma (human)
RARA	RARA - retinoic acid receptor alpha (human)
RXRA	RXRA - retinoid X receptor alpha (human)
SMAD2	SMAD2 - SMAD family member 2 (human)
SMAD3	SMAD3 - SMAD family member 3 (human)
THRB	THRB - thyroid hormone receptor beta (human)
TP53	TP53 - tumor protein p53 (human)
TRHR	TRHR - thyrotropin releasing hormone receptor (human)
TSHR	TSHR - thyroid stimulating hormone receptor (human)
TRPA1	TRPA1 - transient receptor potential cation channel subfamily A member 1 (human)
VDR	VDR - vitamin D receptor (human)

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