Analysis of DNA methylation induced by Particulate matter 10 (PM10)

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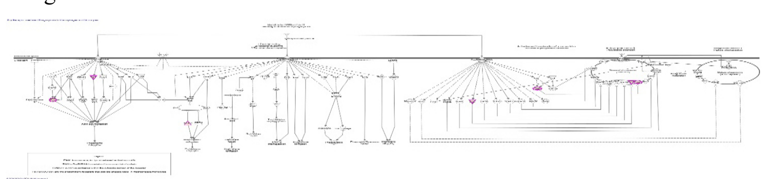
Background/Aims: Fine dust is known to adversely affect various organs as well as the respiratory system, and there are epidemiologic studies that support this. Currently, studies are continuing to reveal the biological mechanism of fine dust, but epigenetic studies are still lacking. In this study, we performed in silico analysis based on microarray results of gene methylation changes after PM10 treatment on respiratory epithelial cells.

Methods: We used five cell lines (BEAS-2B, NCI-H358, HCC-827, A549, NCI-H292), and each cell line was divided into an experimental group treated with PM10 (ERM® CZ120 fine dust) and a control group without treatment. The experimental group was treated with PM10 at a concentration of 50μg/mL for 48 hours. Then we extracted the genomic DNA from the sample and performed CpG microarray (Agilent, Santa Clara, CA, USA). Based on the microarray results, we selected genes with a 2-fold difference in methylation signal. Ingenuity pathway analysis (IPA) was performed with microarray results.

Results: Table 1 shows the result of DNA methylation induced by PM10. CXorf38, NOTCH1, C20orf58, FAM26A, and C1orf45 were identified as top 5 of hypermethylation. RBM22, CHURC1, RANGAP1, HLRC1, and MRPL17 were identified as top 5 of hypomethylation. According to Table 2, cell death and survival, GI disease and cancer, and organismal survival were demethylated, whereas embryonic development, organismal development, system development were methylated. As a result of IPA analysis, it was found that genes related to Fcγ Receptor-mediated phagocytosis in Macrophages and Monocytes pathway were hypermethylated, and genes related to PI3K/AKT signaling pathway were hypomethylated (Table 3). Table 2 lists diseases of functions with an absolute value of activation z score of 2 or more.

Conclusions: Based on the experimental results, it could be inferred that fine dust would silence immune functions by methylating genes involved in Fcγ receptor-medicated phagocytosis in macrophage and monocyte pathways. It can also be inferred that fine dust may be involved in cancer development by activating the PI3K/AKT signaling pathway, known to be associated with cancer, through hypomethylation for related genes



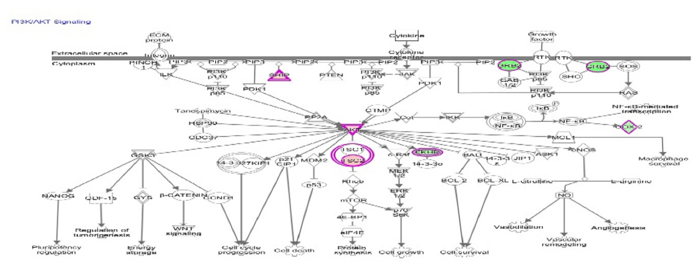


Table 1 DNA methylation induced by PM10

Gene name	Average	A549	BEAS-2B	NCI-H292	NCI-H358	HCC-827
Hypermethylation						
CXorf38	17.123417	7.447684	13.597688	18.863605	36.505558	29.073956
NOTCH1	12.495871	21.300108	15.214883	13.840648	9.066773	7.3197465
C20orf58	10.345367	14.020491	15.933796	14.000034	4.859545	7.633568
FAM26A	8.850058	4.8571386	16.782999	6.9698787	9.429185	10.133997
C1orf45	7.9100084	6.029287	16.169497	7.823332	6.938664	5.851327
Hypomethylation						
RBM22	0.0721161	0.0603412	0.0971302	0.0854014	0.0444359	0.0869777
CHURC1	0.081597	0.1019103	0.0650546	0.1012266	0.0790233	0.0682063
RANGAP1	0.0956569	0.0777072	0.0767674	0.2773797	0.0728453	0.0664457
HLRC1	0.0965133	0.0397702	0.3119337	0.1713778	0.0818249	0.0481367
MRPL17	0.1014527	0.0336548	0.4433059	0.2279839	0.0200131	0.157888

Table 2 Related diseases or functions

Categories	Diseases or Functions Annotation	p-value	Activation z-score	No. of molecules
Demethylation				
Cell Death and Survival	Apoptosis	5.03E-06	-2.780	76
Gastrointestinal Disease, Hepatic System Disease, Organismal Injury and Abnormalities	Liver lesion	3.6E-06	-2.265	129
Cancer, Gastrointestinal Disease, Hepatic System Disease, Organismal Injury and Abnormalities	Liver tumor	3.7E-06	-2.137	125
Organismal Survival	Organismal death	6.01E-12	-2.075	60
Molecular Transport, Protein Trafficking	Transport of protein	0.000383	-2.000	14
Neurological Disease	Neurodegeneration of nerves	0.00266	-2.000	4
Methylation				
Embryonic Development, Organismal Development	Development of head	1.31E-06	2.386	39
Embryonic Development, Organismal Development	Development of body axis	3.6E-07	2.386	42
Embryonic Development, Nervous System Development and Function, Organ Development, Organismal Development, Tissue Development	Formation of brain	0.000093	2.219	21
Cancer, Organismal Injury and Abnormalities, Reproductive System Disease	Breast or gynecological cancer	0.000392	2.219	140
Cellular Growth and Proliferation	Expansion of cells	3.28E-05	2.172	16
Cell Morphology, Embryonic Development	Shape change of embryonic cell lines	0.00185	2.000	4

Table 3 Related ingenuity canonical pathways

Ingenuity Canonical Pathways	-log(p-value)	Ratio	z-score	Molecules
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	2.43	0.0532	2.236	AKT1, ARPC1B, INPP5D, NCK2, VAV3
Adrenomedullin signaling pathway	1.67	0.0305	1.633	ADCY3, ADCY9, AKT1, GRB2, MAPK4, TFAP2C
Cardiac Hypertrophy Signaling	1.31	0.025	1.342	ADCY3, ADCY9, ADRA1A, AKT1, GRB2, NKX2-5
Role of NFAT in Cardiac Hypertrophy	1.52	0.028	1.342	ADCY3, ADCY9, AKT1, CACNA1G, GRB2, NKX2-5
Senescence Pathway	3.03	0.0364	1.265	ACVR1C, AKT1, CBX8, E2F2, EP400, FOXO3, MAPK4, NFATC1, PDK2, RBL2
Synaptogenesis Signaling Pathway	2.63	0.0321	1.265	ADCY3, ADCY9, AKT1, AP2M1, ARPC1B, CDH4, CNTNAP1, EFNB1, GRB2, NECTIN3
CD28 Signaling in T Helper Cells	1.36	0.0331	1.000	AKT1, ARPC1B, GRB2, NFATC1
Estrogen Receptor Signaling	1.99	0.0274	1.000	ADCY3, ADCY9, AKT1, CTBP2, FOXA1, FOXO3, GRB2, MMP17, NOTCH1
PI3K Signaling in B Lymphocytes	1.75	0.0362	1.000	AKT1, FOXO3, INPP5D, NFATC1, VAV3
Reelin Signaling in Neurons	1.35	0.0328	1.000	AKT1, ARPC1B, NECTIN3, PDK2
Opioid Signaling Pathway	1.72	0.0283	0.378	ADCY3, ADCY9, AKT1, AP2M1, CACNA1G, CLTA, MAPK4
Endocannabinoid Neuronal Synapse Pathway	1.88	0.0391	-0.447	ADCY3, ADCY9, CACNA1G, MAPK4, PTGS2
Insulin Receptor Signaling	1.72	0.0357	-0.447	AKT1, FOXO3, GRB2, INPP5D, TSC2
Apelin Endothelial Signaling Pathway	1.43	0.0348	-1.000	ADCY3, ADCY9, AKT1, AQP2
PI3K/AKT Signaling	1.79	0.0324	-1.342	AKT1, FOXO3, GRB2, INPP5D, PTGS2, TSC2