

# 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 50 µg/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-mediated 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

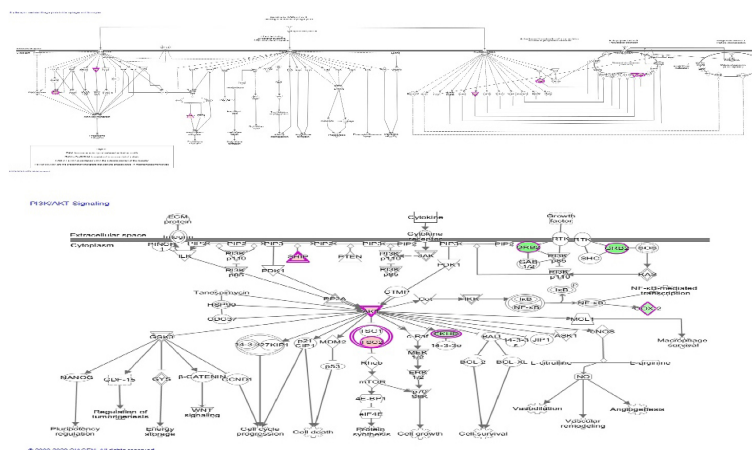


Table 1 DNA methylation induced by PM10

Gene name	Average	BEAS-2B	NCI-H358	HCC-827
Hypermethylation				
CXorf38	47.3247	7.4384	13.9786	16.8056
NOTCH1	42.4597	7.4028	13.9786	16.8056
C20orf58	40.4467	14.0049	14.0049	14.0049
FAM26A	4.0000	4.0000	4.0000	4.0000
C1orf45	4.0000	4.0000	4.0000	4.0000
Hypomethylation				
RBM22	0.00116	0.00116	0.00116	0.00116
CHURC1	0.00116	0.00116	0.00116	0.00116
RANGAP1	0.00116	0.00116	0.00116	0.00116
HLRC1	0.00116	0.00116	0.00116	0.00116
MRPL17	0.00116	0.00116	0.00116	0.00116

Table 2 Related diseases or functions

Categories	Diseases or Functions	p-value	Activation z score	No. of molecules
Demethylation				
Cell Death and Survival	Apoptosis	1.01E-05	-2.380	70
Gastrointestinal Disease	Esophageal Cancer	1.01E-05	-2.380	139
Cancer	Esophageal Cancer	1.01E-05	-2.380	139
Organismal Development	Organismal Development	0.01E-12	-2.070	69
Reproductive System Disease	Reproductive System Disease	0.01E-12	-2.070	69
Protein Trafficking	Protein Trafficking	0.000363	-2.000	14
Neurological Disease	Neurological Disease	0.000363	-2.000	4
Methylation				
Embryonic Development	Embryonic Development	1.31E-05	2.340	39
Organismal Development	Organismal Development	1.31E-05	2.340	42
System Development	System Development	0.000093	2.219	21
Reproductive System Disease	Reproductive System Disease	0.000362	2.219	140
Cellular Growth and Proliferation	Cellular Growth and Proliferation	0.000362	2.219	140
Cell Morphology	Cell Morphology	0.000362	2.219	140
Embryonic Development	Embryonic Development	0.000362	2.219	140

Table 3 Related ingenuity canonical pathways

Ingenuity Canonical Pathways	Log2 fold change	Ratio	Score	Molecules
Phagocytosis in Macrophages and Monocytes	1.67	0.0305	1.533	ADCV3, ADCV9, AKT1, GRB2, MAPK8, MAPK9, PI3K, RAS, RHOA, SRC, TRAF6, TRAF7, TRAF8, TRAF9, TRAF10, TRAF11, TRAF12, TRAF13, TRAF14, TRAF15, TRAF16, TRAF17, TRAF18, TRAF19, TRAF20, TRAF21, TRAF22, TRAF23, TRAF24, TRAF25, TRAF26, TRAF27, TRAF28, TRAF29, TRAF30, TRAF31, TRAF32, TRAF33, TRAF34, TRAF35, TRAF36, TRAF37, TRAF38, TRAF39, TRAF40, TRAF41, TRAF42, TRAF43, TRAF44, TRAF45, TRAF46, TRAF47, TRAF48, TRAF49, TRAF50, TRAF51, TRAF52, TRAF53, TRAF54, TRAF55, TRAF56, TRAF57, TRAF58, TRAF59, TRAF60, TRAF61, TRAF62, TRAF63, TRAF64, TRAF65, TRAF66, TRAF67, TRAF68, TRAF69, TRAF70, TRAF71, TRAF72, TRAF73, TRAF74, TRAF75, TRAF76, TRAF77, TRAF78, TRAF79, TRAF80, TRAF81, TRAF82, TRAF83, TRAF84, TRAF85, TRAF86, TRAF87, TRAF88, TRAF89, TRAF90, TRAF91, TRAF92, TRAF93, TRAF94, TRAF95, TRAF96, TRAF97, TRAF98, TRAF99, TRAF100, TRAF101, TRAF102, TRAF103, TRAF104, TRAF105, TRAF106, TRAF107, TRAF108, TRAF109, TRAF110, TRAF111, TRAF112, TRAF113, 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