

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

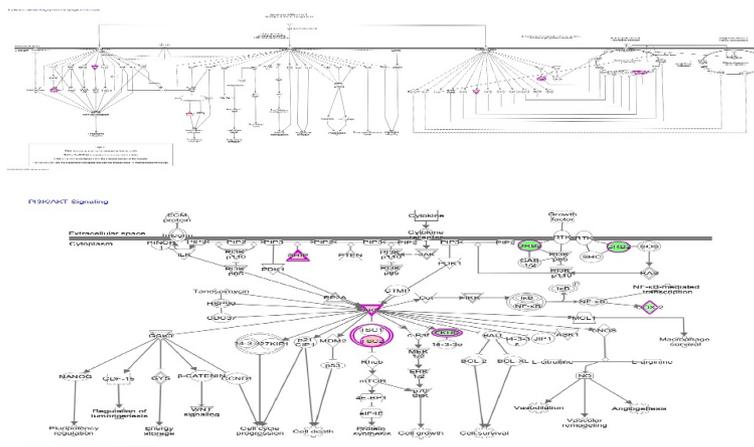


Table 1 DNA methylation induced by PM10

Gene name	Average	BEAS-2B	NCI-H292	NCI-H358	HCC-827
Hypermethylation					
CXorf38	57.3247	7.4384	11.5978	18.8038	8.5095
NOTCH1	52.2691	71.8028	12.2380	19.4884	7.7184
C20orf58	50.3487	18.1041	18.8038	4.8663	7.8384
FAM26A	4.8002	4.8713	18.7029	8.8877	8.4216
C1orf45	4.7808	4.8047	18.8038	7.8384	8.5095
Hypomethylation					
RBM22	0.0119	0.0081	0.0119	0.0081	0.0119
CHURC1	0.0081	0.0081	0.0081	0.0081	0.0081
RANGAP1	0.0081	0.0081	0.0081	0.0081	0.0081
HLRC1	0.0081	0.0081	0.0081	0.0081	0.0081
MRPL17	0.0081	0.0081	0.0081	0.0081	0.0081

Table 3 Related ingenuity canonical pathways

Ingenuity Canonical Pathways	log ₁₀ p-value	Ratio	Z score	Molecules
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes Pathway	0.0032	2.256	2.00	ACT1, APOE, CD14, CD163, CD68, CD80, CD86, CD87, CD88, CD89, CD89L, CD89M, CD89N, CD89O, CD89P, CD89Q, CD89R, CD89S, CD89T, CD89U, CD89V, CD89W, CD89X, CD89Y, CD89Z, CD89AA, CD89AB, CD89AC, CD89AD, CD89AE, CD89AF, CD89AG, CD89AH, CD89AI, CD89AJ, CD89AK, CD89AL, CD89AM, CD89AN, CD89AO, CD89AP, CD89AQ, CD89AR, CD89AS, CD89AT, CD89AU, CD89AV, CD89AW, CD89AX, CD89AY, CD89AZ, CD89BA, CD89BB, CD89BC, CD89BD, CD89BE, CD89BF, CD89BG, CD89BH, CD89BI, CD89BJ, CD89BK, CD89BL, CD89BM, CD89BN, CD89BO, CD89BP, CD89BQ, CD89BR, CD89BS, CD89BT, CD89BU, CD89BV, CD89BW, CD89BX, CD89BY, CD89BZ, CD89CA, CD89CB, CD89CC, CD89CD, CD89CE, CD89CF, CD89CG, CD89CH, CD89CI, CD89CJ, CD89CK, CD89CL, CD89CM, CD89CN, CD89CO, CD89CP, CD89CQ, CD89CR, CD89CS, CD89CT, CD89CU, CD89CV, CD89CW, CD89CX, CD89CY, CD89CZ, CD89DA, CD89DB, CD89DC, CD89DD, CD89DE, CD89DF, CD89DG, CD89DH, CD89DI, CD89DJ, CD89DK, CD89DL, CD89DM, CD89DN, CD89DO, 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CD89IK, CD89IL, CD89IM, CD89IN, CD89IO, CD89IP, CD89IQ, CD89IR, CD89IS, CD89IT, CD89IU, CD89IV, CD89IW, CD89IX, CD89IY, CD89IZ, CD89JA, CD89JB, CD89JC, CD89JD, CD89JE, CD89JF, CD89JG, CD89JH, CD89JI, CD89JJ, CD89JK, CD89JL, CD89JM, CD89JN, CD89JO, CD89JP, CD89JQ, CD89JR, CD89JS, CD89JT, CD89JU, CD89JV, CD89JW, CD89JX, CD89JY, CD89JZ, CD89KA, CD89KB, CD89KC, CD89KD, CD89KE, CD89KF, CD89KG, CD89KH, CD89KI, CD89KJ, CD89KL, CD89KM, CD89KN, CD89KO, CD89KP, CD89KQ, CD89KR, CD89KS, CD89KT, CD89KU, CD89KV, CD89KW, CD89KX, CD89KY, CD89KZ, CD89LA, CD89LB, CD89LC, CD89LD, CD89LE, CD89LF, CD89LG, CD89LH, CD89LI, CD89LJ, CD89LK, CD89LL, CD89LM, CD89LN, CD89LO, CD89LP, CD89LQ, CD89LR, CD89LS, CD89LT, CD89LU, CD89LV, CD89LW, CD89LX, CD89LY, CD89LZ, CD89MA, CD89MB, CD89MC, CD89MD, CD89ME, CD89MF, CD89MG, CD89MH, CD89MI, CD89MJ, CD89MK, CD89ML, CD89MN, CD89MO, CD89MP, CD89MQ, CD89MR, CD89MS, CD89MT, CD89MU, CD89MV, CD89MW, CD89MX, CD89MY, CD89MZ, CD89NA, CD89NB, CD89NC, CD89ND, CD89NE, CD89NF, CD89NG, 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Table 2 Related diseases or functions

Categories	Diseases or Functions Annotation	p-value	Activation z score	No. of molecules
Demethylation	Cell Death and Survival	0.00000	2.380	70
	Cancer-Related Diseases - Hepatic	0.00000	-2.380	70
	Cancer-Related Diseases - Organismal Injury and Abnormalities	0.00000	-2.380	70
	Cancer-Related Diseases - Organismal Survival	0.00000	-2.380	70
	Female System Organismal Injury and Abnormalities	0.00000	-2.380	70
	Cancer-Related Diseases - Organismal Survival	0.00000	-2.380	70
	Organismal Injury and Abnormalities	0.00000	-2.380	70
	Female System Organismal Injury and Abnormalities	0.00000	-2.380	70
	Organismal Injury and Abnormalities	0.00000	-2.380	70
	Female System Organismal Injury and Abnormalities	0.00000	-2.380	70
Methylation	Embryonic Development	0.00000	2.380	70
	Organismal Development	0.00000	2.380	70
	Embryonic Development	0.00000	2.380	70
	Organismal Development	0.00000	2.380	70
	Embryonic Development	0.00000	2.380	70
	Organismal Development	0.00000	2.380	70
	Embryonic Development	0.00000	2.380	70
	Organismal Development	0.00000	2.380	70
	Embryonic Development	0.00000	2.380	70
	Organismal Development	0.00000	2.380	70