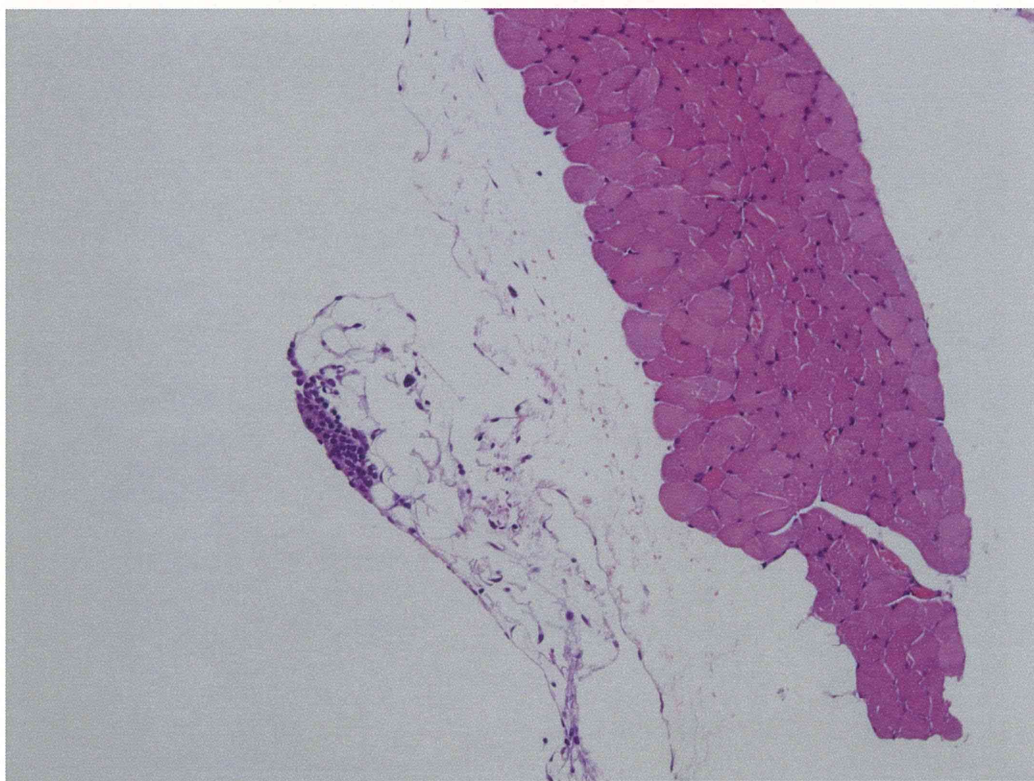
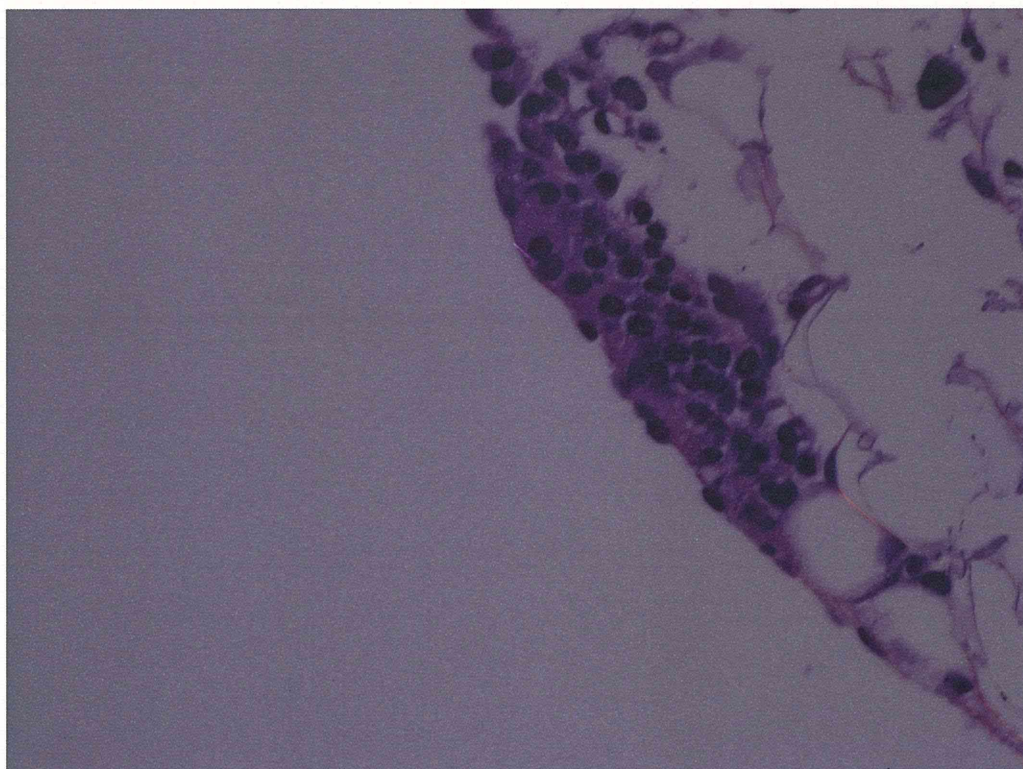


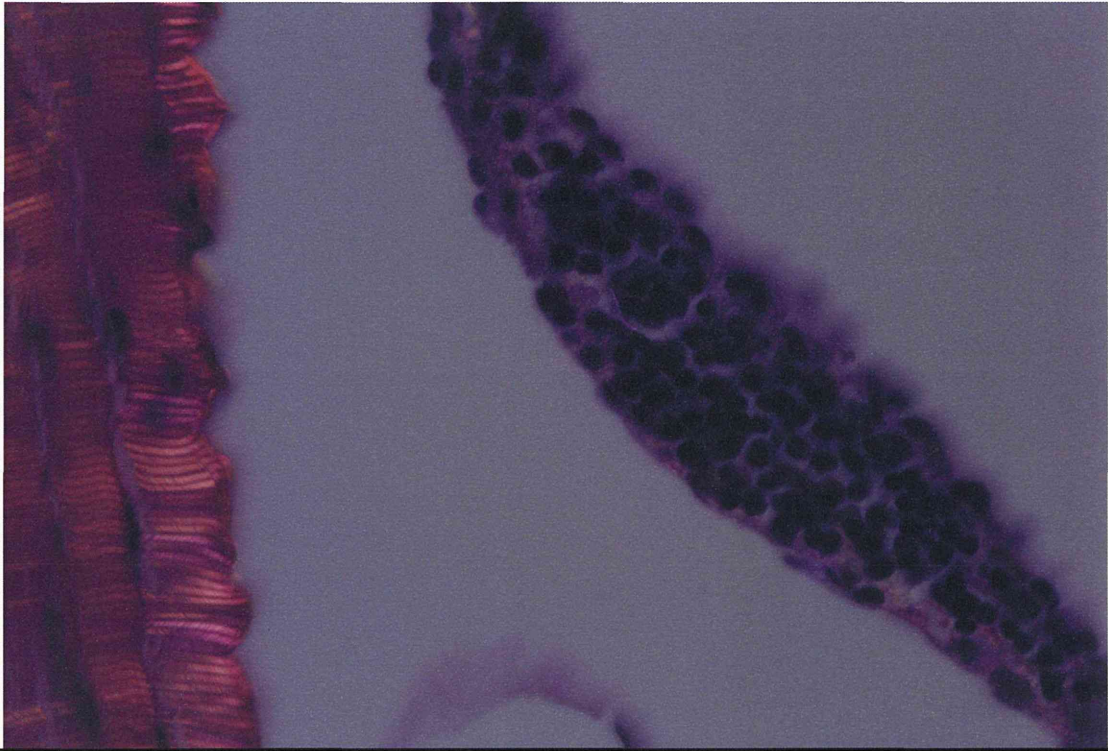
吸入終了後13週目(L #249)



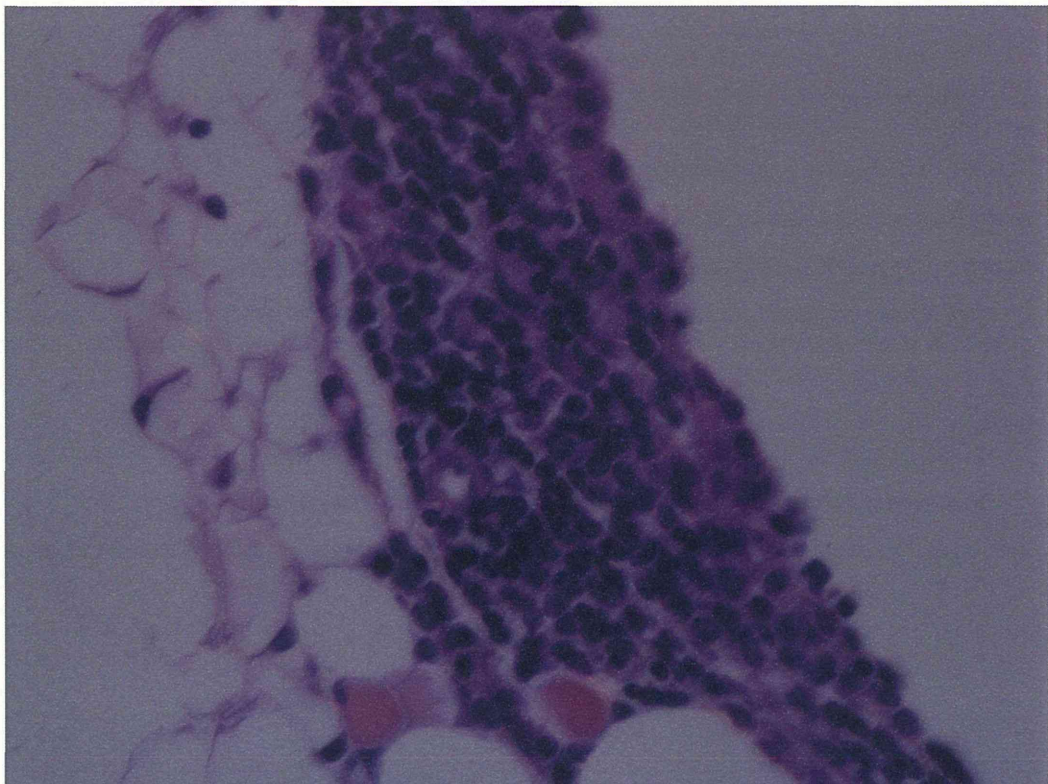
吸入終了後13週目(L #249)



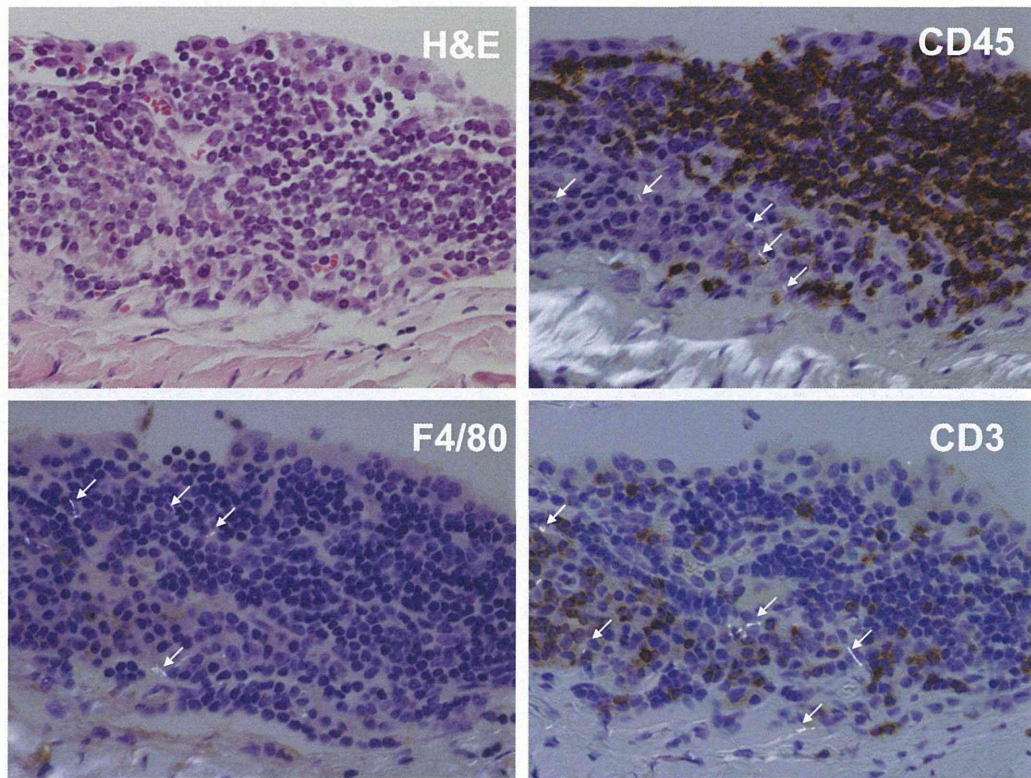
吸入終了後13週目(L #249)



吸入終了後13週目(L #249)



【参考:MWCNT腹腔内投与1年目 MWCNT貪食Mφを伴う慢性炎症巣+反応性中皮】



まとめ

- T-CNTは凝集体・凝固体が少なく、単離した線維成分が多い。気相においても分散性が保たれている。CNTの凝集はファンデルワールス力によるものであるが、凝集体・凝固体が含まれないことが分散性を確保するためには重要であると考えられる。
- T-CNTは、平均長が8 μ m、 3×10^6 本/ μ gの繊維数であると結論した。
- 肺に取り込まれたMWCNTの性状は、気相に分散したT-CNTと同様であった。
- 1日2時間、5日間の反復全身暴露吸入で肺に取り込まれたMWCNTの質量は約3 μ g/lungと考えられた。
- 吸入暴露直後の肺の光学顕微鏡による病理組織検査により、凝集体・凝固体は観察されず、単離繊維が気管支上皮の粘液層に小集合としてトラップされている像、及び単離繊維が肺胞内にまで到達しているのが観察された。好中球浸潤像は弱く、肺胞内のMWCNTはもっぱらマクロファージに貪食されて存在していた。
- 全身吸入暴露後、13週目の所見として、
 - 肺: 異物肉芽腫を認めない。MWCNT貪食Mφを肺胞内に認める。
 - 胸膜面(横隔膜面): MWCNT貪食Mφを伴う円形細胞小集簇を認め、そこを被覆する中皮の立方上皮化を時に認める。
- 今後T-CNTを反復全身暴露吸入したマウスの経時的な組織沈着量の推移を明らかにし、肺病変との関係を明らかにする計画である。

2013年10月18日 今井田班会議

DNAマイクロアレイを用いた遺伝子発現 変動解析

国立医薬品食品衛生研究所
安全性生物試験研究センター 毒性部室長

高木篤也

背景

MWCNT気管内投与ラット肺のマイクロアレイ 解析

(ナノマテリアルのヒト健康影響の評価手法に関する総合研究-H20-化学-一般-006
福島班)

目 的

ナノマテリアルの生体障害作用を分子レベルで明らかにするため、長野班員との共同で、gpt deltaラット雄にナノマテリアルとしてMWCNTを単回経気道曝露後、1ヶ月と3ヶ月後の肺を対象とした定量的なマイクロアレイ解析（Percellome法）を行なった。

Experimental protocol

- ・ Animal : male gpt delta rat (13 weeks old) SLC
- ・ Chemicals : MWCNT (Mitsui, MWCNT-7)
直径80-110nm、長さ 5-20 μ mが \approx 27.5%
Quartz (US Silica社, MIN-U-SIL5) 直径1.6 μ m
- ・ Vehicle : 0.1%Tween80, PBS
- ・ Administration: single i.t.
- ・ Dose: MWCNT (40 or 160 μ g/kg), Quartz (160 μ g/kg)
- ・ Sampling : 1 or 3 M
- ・ Microarray analysis : AFFYMETRIX, Gene Chip Rat Genome 230 2.0
Array and “Percellome” method

病理所見 (1M)

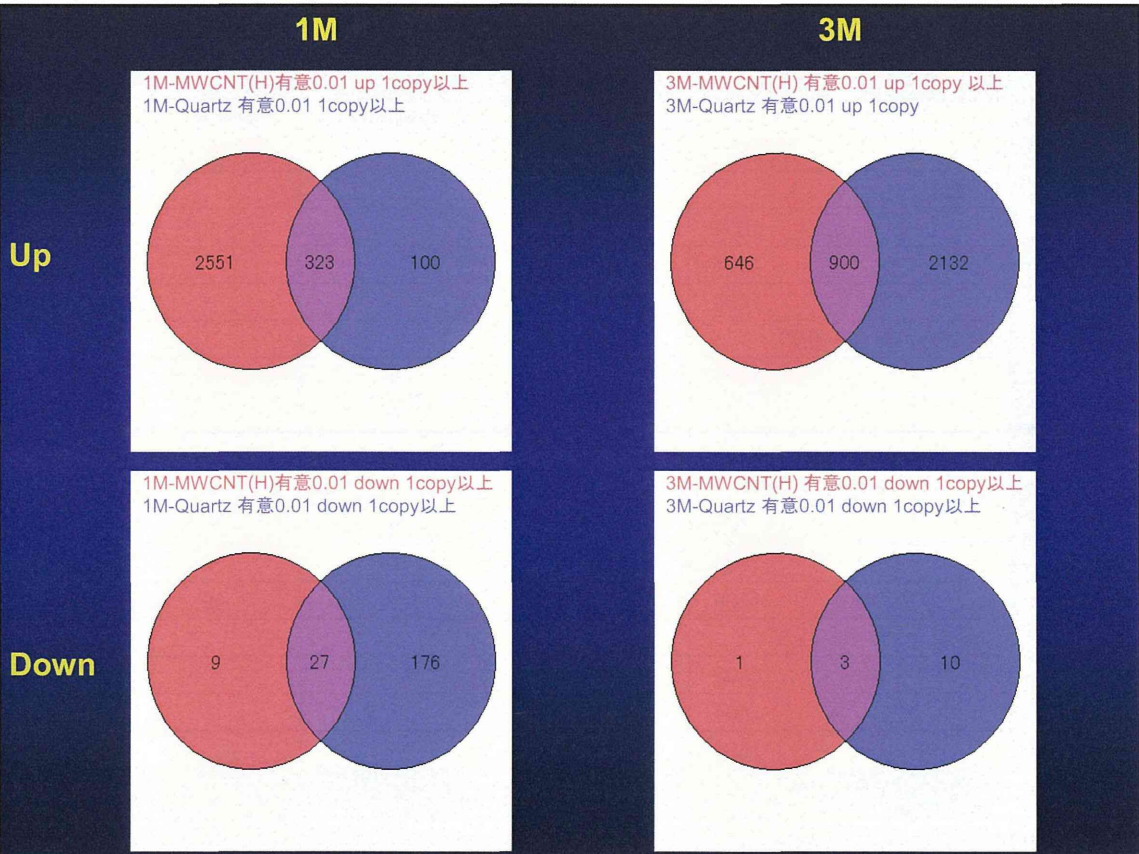
	肺胞マクロ ファージの浸潤	Ⅱ型細胞増生	肉芽形成
対照群			
1001			
1003			
1005			
MWCNT 40 μ g/匹群			
1103	+	+	
1105	+	+	
1107	+	+	
MWCNT 160 μ g/匹群			
1201	+	+	+
1205	+	+	+
1207	+	+	+
Quartz 160 μ g/匹群			
1303	+		
1305	+		
1307	+		

病理所見 (3M)

	肺胞マクロ ファージの浸潤	Ⅱ型細胞増生	肉芽形成
対照群			
1002			
1004			
1006			
MWCNT 40 μ g/匹群			
1102	+	+	
1104		+	
1106	+	+	
MWCNT 160 μ g/匹群			
1202	+	+	+
1204	+	+	+
1206	+	+	+
Quartz 160 μ g/匹群			
1302	+		
1304	+	+	
1306	+	+	

変動遺伝子数 ($p < 0.05$, 1 copy以上)

	up	down
MWCNT(L) 1M	820	10
MWCNT(H) 1M	2874	36
Quartz 1M	423	203
MWCNT(L) 3M	211	13
MWCNT(H) 3M	1546	4
Quartz 3M	3032	13



Gene ontology 解析 (1M)

1M MWCNT & Quartz		1M MWCNT		1M Quartz	
transport	27	transport	188	translation	13
translation	20	metabolic process	123	transport	8
signal transduction	19	regulation of transcription, DNA-dependent	120	metabolic process	7
metabolic process	18	signal transduction	117	regulation of transcription, DNA-dependent	7
immune response	18	protein transport	80	cell differentiation	6
regulation of transcription, DNA-dependent	13	intracellular protein transport	68	multicellular organismal development	6
proteolysis	11	protein amino acid phosphorylation	68	biotin biosynthetic process	5
transcription	10	transcription	65	apoptosis	4
cell adhesion	9	translation	65	cell-cell signaling	4
inflammatory response	9	electron transport	63	signal transduction	4
intracellular protein transport	9	proteolysis	63	synaptic transmission	4
lipid metabolic process	9	ion transport	58	transcription	4
cell proliferation	7	intracellular signaling cascade	51	RNA splicing	3
chemotaxis	7	apoptosis	48	cell adhesion	3
carbohydrate metabolic process	7	immune response	47	cell cycle	3
electron transport	6	vesicle-mediated transport	47	cell cycle arrest	3
ion transport	6	regulation of transcription	42	cell proliferation	3
small GTPase mediated signal transduction	6	cell proliferation	39	electron transport	3
protein amino acid phosphorylation	6	carbohydrate metabolic process	38	induction of apoptosis	3
antigen processing and presentation	6	cell adhesion	38	ion transport	3
multicellular organismal development	5	cell cycle	36	nervous system development	3
apoptosis	5	protein complex assembly	35	regulation of transcription from RNA polymerase II promoter	3
protein transport	5	small GTPase mediated signal transduction	35	small GTPase mediated signal transduction	3
intracellular signaling cascade	5	ubiquitin cycle	35	ATP synthesis coupled proton transport	2
protein complex assembly	5	negative regulation of cell proliferation	34	body fluid secretion	2
ubiquitin cycle	5	multicellular organismal development	33	brain development	2
fatty acid metabolic process	5	inflammatory response	32	cholesterol transport	2
defense response	5	lipid metabolic process	31	cobalamin biosynthetic process	2
cell-cell signaling	4	cell motility	28	dendrite development	2
cell cycle	4	protein folding	28	endocytosis	2
protein modification process	4	anti-apoptosis	27	generation of precursor metabolites and energy	2
response to drug	4	induction of apoptosis	27	immune response	2
cell motility	4	cell differentiation	26	inflammatory response	2
positive regulation of cell proliferation	4	nervous system development	26	lactation	2
neutrophil chemotaxis	4	G-protein coupled receptor protein signaling pathway	24	lung development	2
G-protein coupled receptor protein signaling pathway	4	protein modification process	24	mRNA processing	2

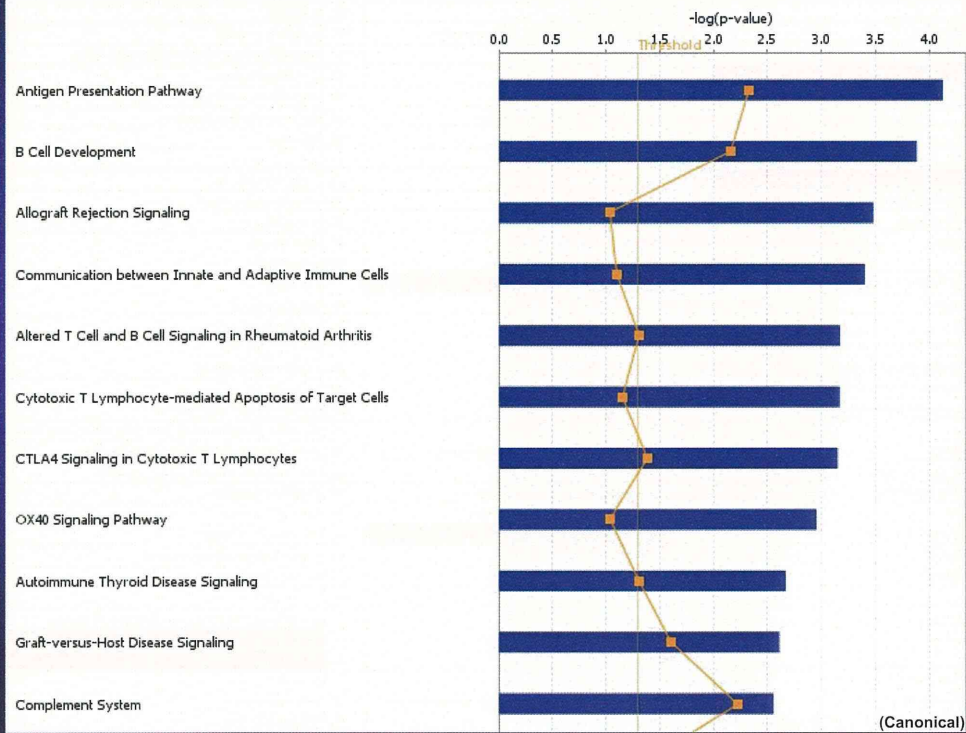
Gene ontology 解析 (3M)

3M MWCNT & Quartz		3M MWCNT		3M Quartz	
transport	72	transport	50	transport	147
regulation of transcription, DNA-dependent	46	regulation of transcription, DNA-dependent	37	regulation of transcription, DNA-dependent	114
signal transduction	45	metabolic process	28	signal transduction	113
metabolic process	38	protein transport	28	metabolic process	86
protein transport	30	transcription	24	translation	84
protein amino acid phosphorylation	26	signal transduction	23	transcription	61
electron transport	26	intracellular protein transport	19	protein amino acid phosphorylation	57
transcription	24	protein amino acid phosphorylation	18	protein transport	53
proteolysis	23	vesicle-mediated transport	17	electron transport	52
intracellular protein transport	23	proteolysis	16	immune response	49
ion transport	23	intracellular signaling cascade	15	proteolysis	47
multicellular organismal development	23	cell cycle	14	intracellular signaling cascade	46
immune response	21	electron transport	13	intracellular protein transport	42
apoptosis	19	translation	13	apoptosis	41
translation	18	small GTPase mediated signal transduction	12	ion transport	41
nervous system development	17	apoptosis	11	cell adhesion	40
cell adhesion	16	cell adhesion	11	multicellular organismal development	40
cell proliferation	16	induction of apoptosis	10	cell proliferation	40
cell cycle	16	regulation of transcription	10	protein folding	35
ubiquitin-dependent protein catabolic process	16	ER to Golgi vesicle-mediated transport	9	cell cycle	33
small GTPase mediated signal transduction	15	ion transport	9	lipid metabolic process	33
ubiquitin cycle	14	mRNA processing	9	ubiquitin cycle	32
cell differentiation	14	RNA splicing	8	small GTPase mediated signal transduction	31
cell motility	14	multicellular organismal development	8	vesicle-mediated transport	30
regulation of transcription	14	transcription from RNA polymerase II promoter	8	carbohydrate metabolic process	29
regulation of apoptosis	14	ubiquitin cycle	8	inflammatory response	29
lipid metabolic process	13	cell proliferation	7	anti-apoptosis	29
carbohydrate metabolic process	13	lipid metabolic process	7	cell differentiation	27
protein modification process	13	negative regulation of transcription	7	positive regulation of cell proliferation	27
G-protein coupled receptor protein signaling pathway	13	protein folding	7	negative regulation of cell proliferation	25
protein folding	12	protein targeting	7	protein modification process	25
vesicle-mediated transport	12	regulation of translation	7	G-protein coupled receptor protein signaling pathway	25
inflammatory response	12	cell differentiation	6	cell motility	24
anti-apoptosis	12	cell division	6	response to stress	24
negative regulation of cell proliferation	11	cell motility	6	regulation of transcription	22

Pathway 解析 (1M MWCNT & Quartz)

Analysis: 1M up and - 2010-12-16 04:07 午後

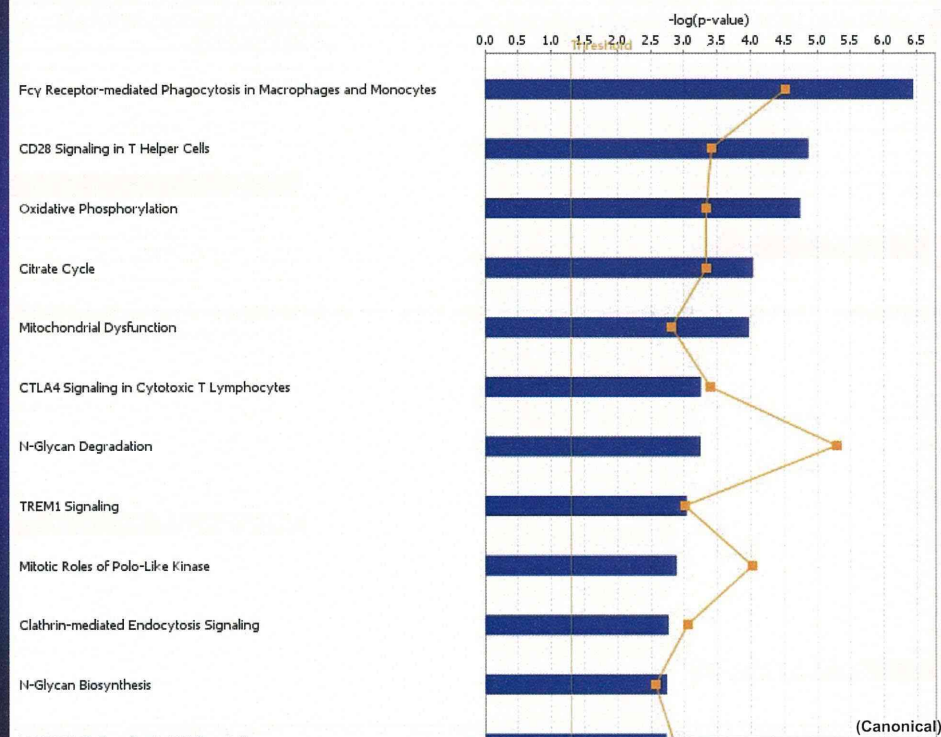
■ 1M up and - 2010-12-16 04:07 □ □ Ratio



Pathway 解析 (1M MWCNT)

Analysis: 1M Mw sub - 2010-12-16 04:16 午後

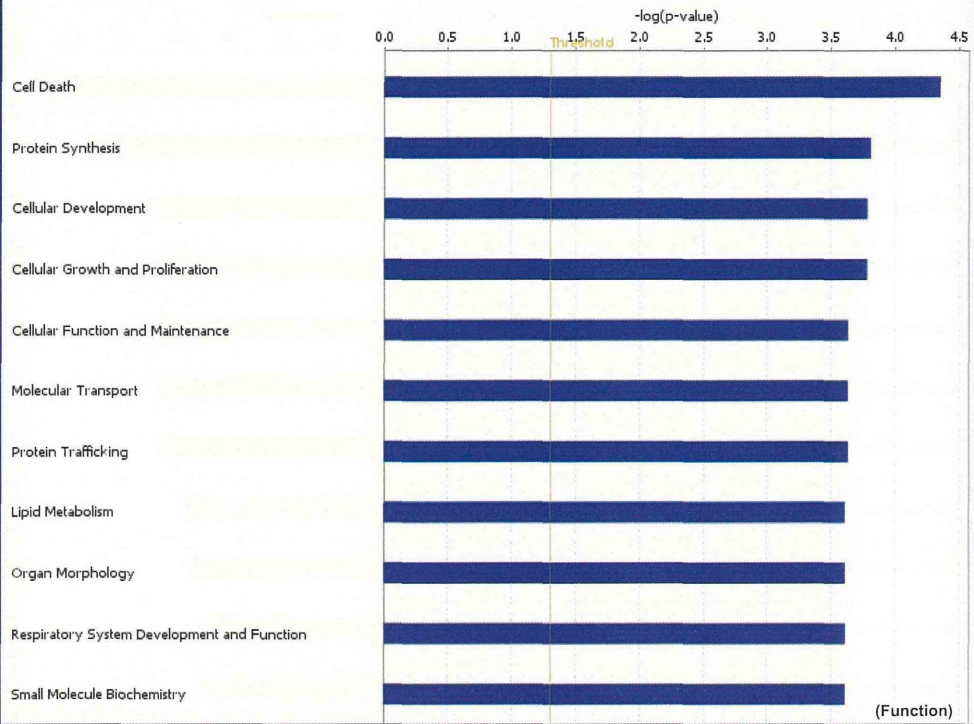
■ 1M Mw sub - 2010-12-16 04:16 □ □ Ratio



Pathway 解析 (1M Quartz)

Analysis: 1M quartz sub - 2010-12-16 04:30 午後

■ 1M quartz sub - 2010-12-16 04:30 □□



Pathway 解析 (3M MWCNT & Quartz)

Analysis: 3M up MW & Q - 2010-12-16 04:40 午後

■ 3M up MW & Q - 2010-12-16 04:40 □□ ◆ Ratio

