厚生労働科学研究費補助金（化学物質リスク研究事業）
（分担）研究報告書

IMR-32 細胞でのメチル水銀に対する遺伝子発現のプロファイリング

分担研究者　黄　基旭　東北大学大学院薬学研究科助手

メチル水銀による毒性発現機構とそれに対する防御機構を解明することを目的として、DNA マイクロアレイ法を用いてメチル水銀によって発現量が変動する遺伝子の検索を行った。その結果、メチル水銀処理により発現量が上昇する遺伝子が 74 種、また発現量が減少する遺伝子が 86 種見つかった。その中にはシグナル伝達、腫瘍形成、ストレス応答および神経変性疾患に関わる遺伝子が多く含まれていた。特にメチル水銀処理によって抗酸化酵素やシグナル伝達に関わる因子をコードする遺伝子や、アルツハイマー病の神経変性過程に関わる calpain/p35/Cdk5 カスケードの関連遺伝子および APBA2 などの発現量が顕著に上昇したことはメチル水銀による特異的な神経細胞死を引き起こす機構の解明において非常に興味深い。

A. 研究目的

メチル水銀で細胞を処理すると、細胞内ではこれに応答した様々な反応が生じ、その結果の一つとして特定な遺伝子の発現量の変動が引き起こされると予想される。そこで、メチル水銀に対する細胞応答を検討するうえでの手がかりを得ることを目的として、神経腫細胞（IMR-32）を用いてメチル処理により発現量の変動する遺伝子を DNA マイクロアレイ法により検索した。

B. 研究方法

1. メチル水銀で処理した IMR-32 細胞から総 RNA の抽出

IMR32 細胞を 5x10^6 cells/900 μl/well になるように 6-well plate に播き、37℃、5% CO_2 存在下で 24 時間培養後、塩化メチル水銀（final 1 μM）を含む滅菌水をそれぞれ 100 μl/well ずつ添加して 6 時間または 24 時間培養した。培養終了後、各 well の培地を取り除き、TRIzol (Invitrogen) 1 ml を加えて細胞を回収した。得られた細胞混合液に 0.2 ml のクロロホルムを加えて激しく撹拌し、室温で 3 分間放置後、12,000 xg

で 15 分間遠心分離した。分離した水層 0.5 ml に同量のイソプロパノールを加えて混和後、室温で 10 分間放置し、12,000 xg で 10 分間遠心分離し、沈殿した総 RNA 画分を得た。この沈殿を 75%エタノール溶液で洗浄し、乾燥後 molecular grade water に溶解し、260 nm の吸光度値から RNA 濃度を算出した。

2. DNA マイクロアレイ法

プローブの作製

総 RNA 250 μg より Oligotex™-dT30 <Super> mRNA Purification kit (From Total RNA) (Takara)を用いて mRNA を精製した。この精製した mRNA 1 μg と 0.5 μg/μl oligo の dT primer (Gibco-BRL) 1 μl を混合し RNase (ribonuclease) free の滅菌蒸留水を加え、全量を 7 μl にし、70℃で 5 分間処理した後に 42℃で 2 分間インキュベートした。この溶液に 5 x Super Script II Reverse Transcriptase (以下 SSII と略す) buffer (Gibco-BRL) を 4 μl、dNTP mixture (2 mM dTTP, 5 mM dATP, dGTP, dCTP) (Gibco-BRL) を 2 μl、100 mM DDT (Gibco-BRL) を 2 μl、40 U RNase Inhibitor (東洋紡) を 2.5 μl、1 mM Fluorilink Cy3-dUTP あるいは Fluorilink Cy5-dUTP (Amersham pharmacia biotech) を 2 μl を加え、RNase free の滅菌蒸留水で全量を 19.5 μl にし、SSII (200 U) を 1 μl 加え、42℃で 40 分間インキュベートした。その後さらに SSII を 1 μl 加えて再度 42℃で 40 分間インキュベートした。インキュベート終了後、滅菌蒸留水を 20 μl、0.5 M EDTA 5 μl、1 M NaOH 10 μl を加え 65℃で 1 時間インキュベートし、1 M Tris-HCl (pH 7.5) 25 μl を加えて中和した。この蛍光標識 cDNA プローブを Microcon-30 (Millipore) で 10~20 μl に濃縮した。この溶液に 250 μl の TE buffer (pH 8.0) を加え、さらに 10~20 μl に濃縮した。この操作を 3 回繰り返して最終的に 18.5 μl になるように調整した。

ハイブリダイゼーション

上記で調整したプローブに 20 x SSC 6.25 μl (最終濃度 5 x SSC) を加え、95℃で 2 分間加熱して変性させた後に、室温で 10 分間放置した。その後、最終濃度が 0.2%になるように SDS を加え、Affymetrix Human Genome Focus 上に溶液をのせ、カバーグラスを被せて湿度の高い状態で 65℃、14 時間インキュベートした。この DNA チップを 2 x SSC-0.1% SDS 溶液に浸し、カバーグラスをはずした。2 x SSC-0.1% SDS 溶液に浸し室温で 20 分間、次いで 0.1% SDS 溶液に浸して室温で 20 分間、0.2 x SSC-0.1% SDS

溶液にて45℃、10分間洗浄した後、0.2xSSC、0.05xSSCで軽く洗浄し、100 x g 、20秒間遠心した後に、室温で乾燥した。このDNAチップの解析は受託した。

（倫理面への配慮）

本研究では動物などは使用せず、生物としてヒト培養細胞のみを用いる。したがって、倫理面での配慮を必要としない。

C. 結果・考察

1. 短時間（6時間）のメチル水銀処理により発現量が変動する遺伝子

DNA マイクロアレイを用いて、IMR-32 細胞を塩化メチル水銀（1 μM）で6時間（生存率ほぼ100%）処理した際に発現量が変動する遺伝子の検索を行った。その結果、6時間のメチル水銀処理によって発現量がメチル水銀未処理時に対して 250％以上上昇する遺伝子が 43 種、また発現量が 50%以下に減少する遺伝子が 43 種見つかった（Tables 1～4）。短時間のメチル水銀処理によって発現量が顕著に上昇した遺伝子の中には、様々なストレスに対して防御作用を示すGCLM、HMOX1 および NQO1 や、シグナル伝達に関わる ETV5、CDKN1A および FZD1、遺伝子の転写調節に関わる ID4 および SFRS11 などが含まれていた（Table 2）。生体は様々な抗酸化酵素やシグナル伝達に関わる因子の発現量を誘導することによってメチル水銀毒性に対して防御作用を示すと思われる。また、メチル水銀によって引き起こされる細胞毒性発現機構の一つとして酸化的ストレスの関与が示されており（Yees. and Choi B.H., 1996 ; Naganuma A. et al, 1998）、それによる細胞死はアポトシスによるものであることが知られている（Dare E. et al, 2000）。今回の DNA マイクロアレイの結果でもメチル水銀処理によって多くの抗酸化酵素やシグナル伝達に関わる因子をコードする遺伝子の発現量が上昇しており、メチル水銀毒性発現への酸化ストレスの関与を強く支持している。一方、様々な神経変性疾患に関わるいくつかの遺伝子の発現量がメチル水銀処理によって上昇することが認められた。特にその中には、アルツハイマー病の神経変性過程に関わる calpain/p35/Cdk5 カスケードの活性化因子である CDK5 およびその結合因子である PLXNA2 が含まれていた。坂上らによって小脳の初代培養細胞系において calpain/p35/Cdk5 カスケードがメチル水銀毒性発現に関わっている可能性（Sakaue M. et al ; 2005）が示されており、calpain/p35/Cdk5 カスケードがメ

チル水銀によって活性化されるならば、メチル水銀による神経細胞に特異的に細胞死を引き起こす機構が説明できる可能性が考えられる。

次に、メチル水銀処理によって発現量が顕著に減少した遺伝子の中にはDNA 損傷の修復などに関わる RPGR、OGG1、EZH1 および LIG1 や、神経変性疾患などに関わる CASP6 および BCHE や、シグナル伝達に関わる MPP2 および WIG1 が含まれていた（Table 4）。酸化ストレスは DNA 損傷を引き起こすことが知られており、メチル水銀による酸化ストレス誘導は DNA 損傷を伴う可能性が考えられる。一方、生体には DNA 損傷に対する多様な修復機構が存在するが、メチル水銀は DNA 損傷の修復に関与する因子の発現を抑制することで細胞毒性を引き起こしている可能性が考えられる。また、生体には様々なストレスより生体を防御するためのシグナル伝達機構が存在する。特に我々は酵母を用いたメチル水銀毒性発現機構の解析を行い、パルミトイル基結合酵素の Akr1 を介したシグナル伝達経路がメチル水銀毒性の軽減に関与していることが明らかにしている。短時間のメチル水銀処理によって発現量の減少が認められた遺伝子の中にはパルミトイル化を介してシグナル伝達に関わる MPP2 や MPP5 が含まれていた（Table 3）。このことから、メチル水銀毒性発現においてパルミトイル化によるシグナル伝達経路は何らかの役割を果たしていると思われる。また、短時間のメチル水銀処理によって発現量が減少した遺伝子の中には細胞内での蛋白質輸送システム関わる遺伝子（BET1、EDH1、GOLGA2、STX10、STX4A）が多く存在した（Table 3）。以前、我々は酵母でのエンドソームを介した液胞への蛋白質輸送システムがメチル水銀毒性を増強していることを報告した。このことより、細胞内での蛋白質輸送システムがヒト培養細胞でも酵母と同様にメチル水銀毒性に関与しているかも知れない。

2. 長時間（24 時間）のメチル水銀処理により発現量が変動する遺伝子

IMR-32 細胞を塩化メチル水銀（1 μM）で 24 時間（生存率ほぼ 90%）処理した際に発現量が変動する遺伝子の検索を行った。その結果、24 時間のメチル水銀処理によって発現量がメチル水銀未処理時に対して 200％以上上昇する遺伝子が 31 種、また発現量が 50%以下に減少する遺伝子が 43 種見つかった（Tables 5～8）。長時間のメチル水銀処理によって発現量が顕著に上昇した遺伝子の中には神経系の形成や伝達に関わる VGF、

ADARB1 およびCTDP1、腫瘍形成に関わるAPBA2 およびEGFR、シグナル伝達に関わる ETV5 および DKK1 などが含まれていた（Table 6）。メチル水銀は中枢神経障害を引き起こすことが知られており、神経系の形成や伝達に関わる遺伝子の発現量の上昇はメチル水銀による毒性発現機構の解明のうえで非常に興味深い。また、長時間のメチル水銀処理によって著しく発現量の上昇が認められたAPBA2 およびEGFR 以外にも腫瘍形成に関わる CDKN1A、IL1A、TNFRSF6B、WIG1 の発現量の上昇が認められた。これらの遺伝子は様々なストレスによる腫瘍形成において重要な役割を果たしており、特にその中CDKN1A およびWIG1 は発癌抑制因子の一つである p53 を介して腫瘍形成を抑制することが知られている（Israeli, D. et al, 1997 ; Kim, T. H. et al, 2000）。一方、メチル水銀処理で顕著な発現量の上昇が認められたAPBA2 はアルツハイマー病の原因物質として知られている amyloid beta の前駆体に結合する蛋白質をコードし、アルツハイマー病の誘発に関与する可能性が示されている（Taru, H. and Suzuki, T., 2004）。メチル水銀によるAPBA2 の発現量の上昇はメチル水銀による中枢神経障害の発症において何らかの影響を与える可能性が考えられる。さらに、長時間のメチル水銀処理によって発現量が上昇した遺伝子の中には短時間のメチル水銀処理時でもその発現量の上昇が認められた遺伝子が含まれていた。その中には、シグナル伝達に関わるETV5、DKK1、FZD1 およびCDKN1A や、遺伝子の転写調節に関わる ID4 および SFRS11 の発現量がメチル水銀処理によって早く上昇し、そのレベルは24 時間まで高く維持されていた（Table 9）。

一方、長時間のメチル水銀処理によって、細胞内での蛋白質輸送に関わるSEC24D、ホルモン調節に関わるPGRMC2、抗酸化酵素の一つであるGLRX、metabolic pathway に関わるFPGTなど多様な機能を示す各々の遺伝子の発現量の減少が顕著に認められた（Table 8）。その中でも、FPGTを含む metabolic pathway に関わる多くの遺伝子（C14orf1、C1orf13、FABP6、GALNS、HIBCH、NAGK、SIAT9）の発現量がメチル水銀処理によって減少した（Table 7）。メチル水銀処理で metabolic psthway に関わる多くの遺伝子の発現量が低下されたことによって、メチル水銀による細胞毒性が引き起こされているかも知れない。しかし、長時間でメチル水銀を処理した細胞は対照細胞に比べて生存率が既に 10%程度低下している

ことから、メチル水銀処理によって細胞毒性が引き起こされた結果、多くのmetabolic psthway に関わる遺伝子の発現が低下された可能性も否定できない。

一方、今回の検討によって短時間および長時間でのメチル水銀処理よって発現量の減少が認められた遺伝子としてchromatin silencingに関わるEZH1、腫瘍形成に関わる CPO などが見出された（Table 9)。さらに、発癌抑制因子の一つである p53 を介して腫瘍形成を抑制することが知られている WIG1 は短時間のメチル水銀処理ではその発現量の減少が認められたのに対し、長時間のメチル水銀処理ではその発現量の上昇が認められた。このことから、メチル水銀毒性の発現において WIG1 が何らかの役割を果たしており、p53 を介したWIG1の抗腫瘍作用もしくは抗apoptpsis作用がメチル水銀毒性の軽減に何らかの関わりがあるかも知れない。

D. 参考文献

Dare E., Gotz M.E., Zhivotovsky B., Manzo L. and Ceccatelli S. (2000) Antioxidants J811 and 17beta-estradiol protect cerebellar granule cells from methylmercury-induced apoptotic cell death. J Neurosci Res. 62, 557-565

Israeli, D., Tessler, E., Haupt, Y., Elkeles, A., Wilder, S., Amson, R., Telerman, A., and Oren, M. (1997) A novel p53-inducible gene, PAG608, encodes a nuclear zinc finger protein whose overexpression promotes apoptosis. EMBO J 16, 4384-4392.

Kim, T. H., Zhao, Y., Barber, M. J., Kuharsky, D. K., and Yin, X. M. (2000) Bid-induced cytochrome c release is mediated by a pathway independent of mitochondrial permeability transition pore and Bax. J Biol Chem 275, 39474-39481.

Naganuma A., Miura K., Tanaka-Kagawa T., Kitahara J., Seko Y., Toyoda H. and Imura N. (1998) Overexpression of manganese-superoxide dismutase prevents methylmercury toxicity in Hela cells. Life Sciences, 62, 157-161

Sakaue M., Okazaki M. and Hara S. (2005) Very low levels of methylmercury induce cell death

of cultured rat cerebellar neurons via calpain activation. Toxicology, 213, 97-106

Taru, H. and Suzuki, T. (2004) Facilitation of stress-induced phosphorylation of beta-amyloid precursor protein family members by X11-like/Mint2 protein. J Biol Chem 279, 21628-21636.

Yee S. and Choi B.H. (1996) Oxidative stress in neurotoxic effects of methylmercury poisoning. Neurotoxicology, 17, 17-26

E. 研究発表

1. 論文発表

なし。

2. 学会発表

なし。

F. 知的財産権の出願・登録状況

なし。

Table 1. 短時間のメチル水銀処理により発現が上昇する遺伝子群（機能による分類）

Functional group	Gene name	% of contro	Function
Neurological disorder	CDK5R1	258.71	Cyclin-dependent kinase 5 regulatory subunit 1, a non-cyclin regulatory subunit for CDK5; cleavage to the constitutively activating form p25 occurs in affected regions of the brain from people with Alzheimer's disease
	HRK	260.77	Harakiri, protein with a putative BH3 domain, interacts with and may inhibit the antiapoptotic activities of BCL2 and BCL-XL (BCL2L1), induces apoptosis, may play a role in apoptotic events in amyotrophic lateral sclerosis (ALS) patients
	MAP1B	261.71	Microtubule-associated protein 1B, neuronal microtubule-binding protein that may play a role in development of the central nervous system, may be involved in the pathogenesis of Lewy bodies associated with Parkinson's disease and dementia
	NEF3	308.2	Neurofilament 3 (150kD medium), a neurofilament component, affects neurofilament organization, increased phosphorylation correlates with neuroblastoma and Alzheimer disease; gene mutation is linked to early onset severe Parkinson disease
	PLXNA2	296.61	Plexin A2, member of the plexin family of semaphorin receptors involved in mediating cell guidance, involved in semaphorin 3 signaling, binds neurophilin-1 (NRP1) and FYN, binds CDK5 through FYN
Signal transduction	ARHGDIB	255.92	Aplysia Ras homolog GDP dissociation inhibitor beta, a hematopoietic cell oriented protein that inhibits dissociation of GDP from the rho subfamily of ras-related proteins; likely involved in apoptosis and the immune response
	CDKN1A	391.33	Cyclin-dependent kinase inhibitor 1A (p21, CIP1, WAF1), induced by p53 (TP53), involved in DNA damage response and repair, cell cycle control, and apoptosis; altered expression may be therapeutic for various cancers and rheumatoid arthritis
	DKK1	284.06	Dickkopf homolog 1 (Xenopus laevis), member of the Dickkopf protein family, ligand for Wnt coreceptor LRP6, inhibits Wnt signaling by blocking Wnt-induced Frizzled (FZD5) and LRP6 complex formation, involved in apoptosis following DNA damage
	ENPP2	277.19	Ectonucleotide pyrophosphatase-phosphodiesterase 2 (autotaxin), multifunctional ecto enzyme and tumor-associated cytokine, hydrolyzes lysophosphatidylcholine to lysophosphatidic acid, promotes cell motility and angiogenesis, enhances tumor aggressiveness

	ETV5	2259.97	Ets variant gene 5 (ets-related molecule), a member of the PEA3 subfamily of the ETS family, acts as a transcriptional activator, activated by JUN and PKA, marker for chronic B-cell leukemia and mantle-cell lymphoma
Signal transduction	FZD1	377.95	Frizzled homolog 1 (Drosophila), member of the frizzled/taste2 receptor family of G protein-coupled receptors, enhances Wnt family-mediated signal transduction
	FZD7	254.11	Frizzled (Drosophila) homolog 7, member of the frizzled/taste2 receptor family of G protein-coupled receptors, predicted to bind Wnt, may contribute to gastric and esophageal cancers by indirectly enhancing beta-catenin (CTNNB1) mediated signaling
	G3BP	268.08	Ras-GTPase-activating protein SH3-domain-binding protein, protein with both DNA and RNA helicase activities, binds GAP (RASA1) in RAS activated cells, may have a role in the regulation of cell proliferation
	HEY1	285.31	Hairy enhancer-of-split related with YRPW motif 1, a transcriptional corepressor of a family of basic helix loop helix (bHLH) proteins, involved in Notch signaling and angiogenesis, may play a role in embryonic development
	PSA	284.61	Kallikrein 3, (prostate specific antigen), serine protease, dissolves seminal coagulum, may play a role in regulation of cell growth, may be involved in prostate cancer invasion and metastasis, used as marker to test for and monitor prostate cancer
	SHB	304.32	Src homology 2 domain containing adaptor protein B, an adapter protein that links signaling proteins and various receptors (including TCR), mediates apoptosis, cell differentiation, CD3-induced IL2 expression, Erk activation (MAPK1, MAPK3) and Ca2+ flux
	STK17A	309.98	Serine threonine kinase 17a, contains an N terminal catalytic domain and C terminal regulatory domain, may play a role in cross resistance against DNA damaging anti-cancer drugs, overproduction induces apoptosis
Stress response	DNAJB4	264.08	DnaJ (Hsp40) homolog subfamily B member 4, member of the DNAJ-like heat shock protein 40 family, a predicted heat shock protein and chaperone whose expression is inducible by heat
	GCLM	892.87	Glutamate-cysteine ligase modifier subunit, involved in the first step of glutathione synthesis and in drug and environmental chemical detoxification, associated with myocardial infarction and a possible predisposition to mesothelioma upon gene mutation
	HMOX1	741.62	Heme oxygenase (decycling) 1, cleaves the heme ring at the alpha methene bridge to form biliverdin and carbon monoxide, involved in oxidative stress response, altered expression is associated with Alzheimer disease, Parkinson disease, and some cancers

	HSPA1A	290.74	Heat shock 70 kDa protein 1A, an HSP70 family chaperone that modulates stress responses; gene polymorphism is associated with ankylosing spondylitis, celiac disease, and rheumatoid arthritis; altered expression is associated with lung cancer and diabetes
Stress response	HSPA1B	298.09	Heat shock 70kDa protein 1B, involved in the heat shock response; gene polymorphism correlates with breast cancer, Celiac disease, type I diabetes, non-Hodgkin's lymphoma, ankylosing spondylitis, systemic lupus erythematosus, and nasopharyngeal carcinoma
	NQO1	471.19	NAD(P)H dehydrogenase quinone 1, cytosolic reductase targeting quinones, predicted to function in detoxification and oxidative stress responses; gene polymorphisms are associated with increased benzene hematotoxicity, urolithiasis and various cancers
Transporter	SLC6A11	272.38	Solute carrier family 6 member 11 (gamma-amino butyric acid (GABA) transporter GAT-3), a high-affinity GABA transporter, activity is specifically inhibited by (S)SNAP 5114, may participate in temporal lobe epilepsy
	SLC7A5	262.99	Solute carrier family 7 member 5, an L-type and neutral amino acid transporter, binds CD98 heavy chain (SLC3A2) to mediate large neutral amino acid transport, increased expression may correlate with disease progression in colon cancer
Tumor progression	IFI16	371.29	Interferon gamma-inducible protein 16, a transcriptional regulator that binds AIM2 and p53 (TP53) and functions in TP53 -mediated apoptosis and cell cycle regulation, downregulated in breast carcinoma and may function in autoimmune disease pathogenesis
	STC1	301.36	Stanniocalcin 1, a glycopeptide hormone that regulates growth, reproductive events, calcium homeostasis, bone and muscle mass and structure, and angiogenesis, may serve as a marker for detection of tumor cells in blood
	TIMP3	292.64	Tissue inhibitor of metalloproteinase 3, involved in extracellular matrix remodeling, has a role in apoptosis; increased expression may be therapeutic for rheumatoid arthritis, breast cancer, and malignant melanoma
Unknown function or poorly characterized	ALCAM	302.06	Activated leukocyte cell adhesion molecule, an immunoglobulin superfamily member and ligand for CD6, involved in hematopoietic cell adhesion, may play a role in osteogenesis, marker for tumor progression in malignant
	BAIAP2	449.9	Brain-specific angiogenesis inhibitor 1-associated protein 2, interacts with many different proteins (such as BAI1, WASF1, CDC42, RAC1, and DRPLA), may be a substrate for INSR, involved in cytoskeletal organization and lamellipodia and filopodia formation

	CXCL12	290.62	Chemokine C-X-C motif ligand 12, an alpha-chemokine ligand for CXCR4, stimulates leukocyte adhesion, migration, and chemotaxis, inhibits infection by lymphocyte-tropic HIV-1, upregulation is associated with metastasis,
	EPM2A	287.36	Epilepsy progressive myoclonus type 2 (laforin), a protein tyrosine-serine-threonine phosphatase, binds glycogen and may play a role in glycogen metabolism; mutations of the corresponding gene result in Lafora's disease, a progressive myoclonus epilepsy
Unknown function or poorly characterized	HSU53209	275.97	Transformer 2 alpha, putative splicing factor that may regulate female-specific gene expression and sexual differentiation
	ID3	298.9	Inhibitor of DNA binding 3, basic helix-loop-helix family member that negatively regulates cell differentiation by inhibiting DNA binding of certain bHLH transcription factors; decreased expression may correlate with
	ID4	655.97	Inhibitor of DNA binding 4 dominant negative helix-loop-helix protein, HLH transcription factor inhibitor, downregulated in gastric adenocarcinoma, may act in breast and ovarian cancer; rat Id4 is strongly upregulated in mammary carcinomas
	NT5C2	251.31	5'-nucleotidase cytosolic II, a regulatory allosteric enzyme of purine nucleotide catabolism, activity is stimulated by glycerate 2,3 bisphosphate and ATP; reduced activity in lymphocytes is associated with systemic lupus erythematosus
	PCDH8	273.83	Protocadherin 8, a member of the protocadherin subfamily of the cadherin superfamily, may mediate cell-cell adhesion, predominantly expressed in
	PDE3B	250.15	cGMP-inhibited phosphodiesterase 3B, activated by phosphatidylinositol 3-kinase, downregulates lipolysis in the insulin receptor pathway, may be involved in cardiovascular tissue responses to cAMP along with cGMP-inhibited phosphodiesterase 3A (PDE3A)
	PGF	271.67	Placenta growth factor, a secreted dimeric angiogenic factor that potentiates the effects of VEGF on vascular endothelial cell proliferation and permeability, may play a role in angiogenesis and pathologic neovascularization
	PIGL	260.91	Phosphatidylinositol glycan class L, a N-acetylglucosaminylphosphatidylinositol de-N-acetylase, likely involved in glycosylphosphatidylinositol anchor biosynthesis
	POLR2A	293.71	RNA polymerase II polypeptide A, the largest subunit of RNA polymerase II, involved in transcriptional initiation and elongation and termination and mRNA cleavage and polyadenylation, putative target of viral proteins involved in viral
	SETMAR	295.92	SET domain and mariner transposase fusion, a putative protein encoded by Hsmar1 mariner transposon that is apparently a product of the splicing of transposase to a cellular gene encoding a SET domain

	SFRS11	435.6	Splicing factor arginine serine rich 11, a pre-mRNA splicing factor containing arginine-serine rich and RRM domains, involved in mRNA splicing, binds to the 65 kDa subunit of the U2 auxiliary splice factor

Table 2. 短時間のメチル水銀処理により発現が上昇する遺伝子(発現変動順)

Gene name	% of control
ETV5	2259.97
GCLM	892.87
HMOX1	741.62
ID4	655.97
NQO1	471.19
BAIAP2	449.90
SFRS11	435.60
CDKN1A	391.33
FZD1	377.95
IFI16	371.29
STK17A	309.98
NEF3	308.20
SHB	304.32
ALCAM	302.06
STC1	301.36

Gene name	% of control
ID3	298.90
HSPA1B	298.09
PLXNA2	296.61
SETMAR	295.92
POLR2A	293.71
TIMP3	292.64
HSPA1A	290.74
CXCL12	290.62
EPM2A	287.36
HEY1	285.31
PSA	284.61
DKK1	284.06
ENPP2	277.19
HSU53209	275.97
PCDH8	273.83

Gene name	% of control
SLC6A11	272.38
PGF	271.67
G3BP	268.08
DNAJB4	264.08
SLC7A5	262.99
MAP1B	261.71
PIGL	260.91
HRK	260.77
CDK5R1	258.71
ARHGDIB	255.92
FZD7	254.11
NT5C2	251.31
PDE3B	250.15

Table 3. 短時間のメチル水銀処理により発現が減少する遺伝子群(機能による分類)

Functional group	Gene name	% of contro	Function
Chromatin remodeling	EZH1	34.59	Enhancer of zeste homolog 1 (Drosophila), a member of the E(z) family of proteins, interacts with the Polycomb-group related protein (EED), may be involved in chromatin silencing
	H1F0	41.33	H1(0)-type member of the H1 histone family involved in compaction of DNA into nucleosomes and into high-order chromatin structures
	SMARCA2	44.1	SWI-SNF related matrix associated actin dependent regulator of chromatin subfamily a member 2, mediates transcriptional regulation by nuclear receptors by remodeling chromatin, controls cell cycle through association with RB1, regulates myogenesis
	TACC2	41.94	Transforming acidic coiled-coil containing protein 2, a putative tumor suppressor that binds microtubules, putative transcription factor GAS41, and the SWI/SNF chromatin remodeling complex, decreased expression correlates with breast neoplasms
G-protein	RAB33A	44.14	Ras-related GTP-binding protein 33a, a putative GTP-binding protein and GTPase
	RAB7L1	42.29	RAB7 member RAS oncogene family-like 1, a putative RAB monomeric GTPase
	RPGR	20.17	Retinitis pigmentosa GTPase regulator, a putative guanyl nucleotide exchange factor involved in vision; gene mutations cause X-linked retinitis pigmentosa type 3, X-linked progressive cone dystrophy, atrophic macular degeneration, and deafness
	RREB1	41.12	Ras responsive element binding protein 1, a transcription activator that binds a distal Ras-responsive element in the calcitonin (CALCA) gene, implicated in the transcriptional activation response to Ras- and Raf-induced cell differentiation
Intracellular trafficking	BET1	45.54	S. cerevisiae Bet1 homolog, may be involved in ER to Golgi transport
	EHD1	49.78	H-domain containing 1, a member of the eps15 homology domain containing family, interacts with synaptosomal protein SNAP29, interacts with IGF1R, represses IGF1 -mediated signaling and may be involved in endocytosis
	GOLGA2	43.99	Golgi autoantigen golgin subfamily a 2 (Golgin 95, Golgi matrix protein 130), interacts with GRASP65 (GOLPH5) and the GTP-bound forms of RAB33B, RAB1, and RAB6A, involved in Golgi transport; autoantigen in some autoimmune disorders

	STX10	39.89	Syntaxin 10, a member of the syntaxin family of proteins involved in regulation of membrane transport, docking, and fusion, may be involved in vesicular transport
	STX4A	48.81	Syntaxin 4, broadly expressed target SNAP receptor (t-SNARE), involved in targeting and exocytosis of a variety of secretory vesicles, interacts with SNAP23, regulates alpha granule release in platelets
Neurological disorder	BCHE	37.68	Butyrylcholinesterase, serum cholinesterase that regulates smooth muscle tone in pulmonary artery and is found in amyloid plaques in Alzheimer's disease; mutations in the gene cause acholinesterasemia and apnea upon succinylcholine administration
	CASP6	37.67	Caspase 6, a cysteine protease that induces apoptosis when activated, mediates lamin cleavage and nuclear fragmentation during apoptosis, cleaves beta amyloid precursor protein (APP) and may play a role in neuronal cell death in Alzheimer disease
	DSCR1	40.63	Down syndrome critical region gene 1 (myocyte-enriched calcineurin interacting protein 1), inhibits calcineurin A (PPP3CA)-dependent pathways, has a role in muscle and CNS development, expression is elevated in Down syndrome and Alzheimer's disease
Signal transduction	MPP2	33.3	Membrane protein palmitoylated 2 (discs-large 2), a palmitoylated membrane protein that contains PDZ, SH3 and guanylate kinase domains and may have roles in signal transduction
	MPP5	50.03	Membrane protein palmitoylated 5 (MAGUK p55 subfamily member 5), binds Crumbs 3 (CRB3) to participate in establishment of polarity in epithelial cells
	RANBP8	43.85	Importin 8 (Ran binding protein 8), interacts with the Ran GTPase (RAN), may be involved in signal transduction
	TRAF5	46.95	TNF receptor-associated factor 5, member of a family of proteins that interact with the cytoplasmic domain of oligomerized TNF receptors, binds CD40 (TNFRSF5) and mediates signaling through activation of NF-kappaB and JNK pathways
	WIG1	39.91	p53 target zinc finger protein, a zinc finger protein that has transient expression which inhibits tumor cell growth, expression is upregulated by DNA damaging agent, mitomycin C
Transporter	SLC1A1	47.33	Solute carrier family 1 member 1, a high affinity glutamate transporter that mediates synaptic reuptake of glutamate, functions in excitatory neurotransmission; mutation of the mouse Slc1a1 gene causes dicarboxylic aminoaciduria

	SLC35A1	49.43	Solute carrier family 35 member (CMP-sialic acid transporter) A1, a transporter present in the Golgi membrane that is important for normal sialylation of glycoproteins and glycolipids, truncated variants cause congenital type IIf glycosylation disorders
Tumor progression	CD83	45.71	CD83 antigen (activated B lymphocytes immunoglobulin superfamily), may play a role in antigen presentation and lymphocyte activation, expressed on dendritic cells at final stage of maturation
	CPO	41.72	rotein containing a zinc carboxypeptidase domain, has moderate similarity to carboxypeptidase B2 (plasma) (mouse Cpb2), which is an acute phase protein that is upregulated in inflammation and inhibits
	MAGE-E1	45.17	Melanoma antigen family E 1 (cancer and testis specific), a member of the MAGE-C family, serves as a tumor antigen associated with various tumors but normally expressed only in testis, may be a potential target for cancer immunotherapy
	OGG1	32.57	8-oxoguanine DNA glycosylase 1, a nuclear and mitochondrial base excision DNA repair enzyme that also has DNA-AP lyase activity; loss and mutations and in the corresponding gene are found in a variety of
Unknown function or poorly characterized	CALD1	41.26	Caldesmon 1, member of the caldesmon family of actomyosin binding proteins, contains myosin, tropomyosin, actin and calmodulin binding domains, binds actomyosin, inhibits myosin MgATPase activity, and is involved in muscle contraction and cell growth
	CGI-57	46.93	Protein of unknown function, has strong similarity to uncharacterized mouse 1810031K17Rik
	DOM3Z	30.34	DOM-3 homolog Z (C. elegans), a nuclear protein with a leucine zipper motif, has similarity to C. elegans DOM-3 which may be involved in germ cell development
	EMILIN	41.45	Protein containing two collagen triple helix repeats and an EMI domain, has moderate similarity to collagen type XXVI alpha 1 (mouse Emid2), which is part of the extracellular matrix and binds mouse Emu1 and mouse Serpinh1
	JWA	46.68	ADP-ribosylation-like factor 6 interacting protein 5, binds CC chemokine receptor 5 (CCR5), and PRAF2
	LIG4	37.57	ATP-dependent DNA ligase IV, acts in nonhomologous end-joining during DNA double-strand break repair and V(D)J recombination; mutations are associated with immunodeficiency syndromes and leukemia, polymorphism correlates with breast cancer
	MDM1	39.44	Protein of unknown function, has high similarity to uncharacterized transformed mouse 3T3 cell double minute 1 (mouse Mdm1)

	MLF1	49.51	Myelodysplasia/myeloid leukemia factor 1, a cytoplasmic protein; gene fusion with NPM (NPM1) in acute myeloid leukemia results in misdirection of the fusion protein to the nucleus
	NDP52	47.15	Nuclear domain 10 protein, contains an extended central coiled-coil domain, a leucine zipper motif, and a putative C-terminal LIM domain, interacts with itself to form homodimers, may play a role in viral life
Unknown function or poorly characterized	OSRF	39.22	Protein containing two tetratricopeptide repeats, which may mediate protein-protein interactions, has strong similarity to uncharacterized mouse 2900001O04Rik
	PCL1	48.68	Prenylcysteine oxidase 1 (prenylcysteine lyase), a lysosomal FAD-dependent thioether oxidase that catalyzes the cleavage of prenylcysteine residues from prenylated proteins to form free cysteine and an isoprenoid product in prenylprotein degradation
	RBPMS	47.7	RNA-binding protein gene with multiple splicing, a protein containing RNA recognition motifs, may play a role in RNA metabolism
	TCEAL1	41.78	Transcription elongation factor A (SII)-like 1, a nuclear phosphoprotein that represses or activates transcription depending on promoter context
	TRO	44.45	Trophinin, an adhesion molecule that forms a complex with tastin (TROAP) and bystin (BYSL) and may play a role in homophilic cell adhesions formed between endometrial epithelia and trophoblast cells during the embryonic implantation process
	TYRP1	43.14	Tyrosinase-related protein 1, catalyzes the oxidation of 5,6-dihydroxyindole-2-carboxylic acid and related compounds in melanin biosynthesis, binds and stabilizes tyrosinase (TYR); gene mutations are associated with oculocutaneous albinism type 3
	ZNF261	41.13	Zinc finger protein 261, member of the MYM zinc-binding family; rearrangement of corresponding gene is associated with nonspecific X-linked mental retardation

Table 4. 短時間のメチル水銀処理により発現が減少する遺伝子（発現変動順）

Gene name	% of control
RPGR	20.17
DOM3Z	30.34
OGG1	32.57
MPP2	33.30
EZH1	34.59
LIG4	37.57
CASP6	37.67
BCHE	37.68
OSRF	39.22
MDM1	39.44
STX10	39.89
WIG1	39.91
DSCR1	40.63
RREB1	41.12
ZNF261	41.13

Gene name	% of control
CALD1	41.26
H1F0	41.33
EMILIN	41.45
CPO	41.72
TCEAL1	41.78
TACC2	41.94
RAB7L1	42.29
TYRP1	43.14
RANBP8	43.85
GOLGA2	43.99
SMARCA2	44.10
RAB33A	44.14
TRO	44.45
MAGE-E1	45.17
BET1	45.54

Gene name	% of control
CD83	45.71
JWA	46.68
CGI-57	46.93
TRAF5	46.95
NDP52	47.15
SLC1A1	47.33
RBPMS	47.70
PCL1	48.68
STX4A	48.81
SLC35A1	49.43
MLF1	49.51
EHD1	49.78
MPP5	50.03

Table 5. 長時間のメチル水銀処理により発現が上昇する遺伝子群(機能による分類)

Functional group	Gene name	% of control	Function
Intracellular trafficking	DNM2	250.98	Dynamin II, member of a family of 100-kD guanosine triphosphatases, regulates budding of endocytic vesicles at the plasma membrane and may function in the formation of transport vesicles at the trans-Golg
	FLJ11116	213.35	WD repeat domain 44 (rab11-binding protein), binds GTP-charged rab11, may play a role in receptor recycling
Neurological disorder	ADARB1	253.52	Double-stranded RNA adenosine deaminase, mediates mRNA editing of glutamate receptor subunit B and the small delta antigen (HDAg S) of hepatitis delta virus (HDV); overexpression may play a role in Down Syndrome phenotype development
	CLN8	222.62	ceroid-lipofuscinosis, neuronal 8 (epilepsy, progressive with mental retardation)
	CTDP1	252.84	Ceroid-lipofuscinosis neuronal 8, a protein that cycles between endoplasmic reticulum and Golgi and may play a role in central nervous system development; gene mutation is detected in ceroid lipofuscinosis with progressive epilepsy and mental retardation
	STMN2	240.5	Superiorcervical ganglia neural specific protein 10, a member of the stathmin family involved in microtubule depolymerization and induced by nerve growth factor; metabolism and compartmentalization may be altered in Alzheimer's disease
	VGF	310.64	VGF nerve growth factor inducible, expressed in neuroendocrine cells
Signal transduction	DKK1	250.12	Dickkopf homolog 1 (Xenopus laevis), member of the Dickkopf protein family, ligand for Wnt coreceptor LRP6, inhibits Wnt signaling by blocking Wnt-induced Frizzled (FZD5) and LRP6 complex formation, involved in apoptosis following DNA damage
	DUSP9	202	Dual specificity phosphatase 9, inactivates mitogen-activated protein kinases via phosphotyrosine and phosphothreonine residue dephosphorylation, acts in MAP kinase signal transduction; may act as a diagnostic and prognostic marker in kidney carcinomas
	ETV5	257.89	Ets variant gene 5 (ets-related molecule), a member of the PEA3 subfamily of the ETS family, acts as a transcriptional activator, activated by JUN and PKA, marker for chronic B-cell leukemia and mantle-cell
	FZD1	238.04	Frizzled homolog 1 (Drosophila), member of the frizzled/taste2 receptor family of G protein-coupled receptors, enhances Wnt family-mediated signal transduction

Stress response	HUS1	223.62	Hydroxyurea sensitive 1, part of a DNA damage-responsive complex that includes RAD1 and RAD and is predicted to function as a DNA clamp loader, participates in DNA checkpoints, and may contribute to ovarian carcinogenesis
Stress response	NQO1	213.5	NAD(P)H dehydrogenase quinone 1, cytosolic reductase targeting quinones, predicted to function in detoxification and oxidative stress responses; gene polymorphisms are associated with increased benzene hematotoxicity, urolithiasis and various cancers
Tumor progression	APBA2	267.12	Amyloid beta precursor protein binding family A member 2, modulates amyloid precursor protein (APP) metabolism and production, may be involved in synaptic vesicle docking and fusion, may play a role in Alzheimer's disease
	CDKN1A	221.69	Cyclin-dependent kinase inhibitor 1A (p21, CIP1, WAF1), induced by p53 (TP53), involved in DNA damage response and repair, cell cycle control, and apoptosis; altered expression may be therapeutic for various cancers and rheumatoid arthritis
	EGFR	250.1	Epidermal growth factor receptor, a receptor protein tyrosine kinase that binds epidermal growth factor (EGF) and transforming growth factor alpha (TGFA), involved in cell proliferation and differentiation, implicated in tumor invasion and metastasis
	ID4	224.23	Inhibitor of DNA binding 4 dominant negative helix-loop-helix protein, HLH transcription factor inhibitor, downregulated in gastric adenocarcinoma, may act in breast and ovarian cancer; rat Id4 is strongly upregulated in mammary carcinomas
	IL1A	206.85	Interleukin 1 alpha, a cytokine that affects cell migration, cell proliferation, and the inflammatory response; inhibition may be therapeutic for rheumatoid arthritis, altered expression may be therapeutic for AIDS and
	TNFRSF6B	234.05	Tumor necrosis factor receptor superfamily member 6b, has multiple splice forms, a helicase-like protein and a decoy receptor, which binds and inhibits Fas (TNFSF6) and LIGHT (TNFSF14)-mediated cell death; overexpressed in some lung, GI, and colon tumors
	WIG1	220.27	p53 target zinc finger protein, a zinc finger protein that has transient expression which inhibits tumor cell growth, expression is upregulated by DNA damaging agent, m鰾tomycin C
Unknown function or poorly characterized	ARSB	201.51	Arylsulfatase B (N-acetylgalactosamine 4-sulfatase), hydrolyzes the sulfate ester group from N-acetylgalactosamine 4-sulfate residues of dermatan sulfate; genetic mutation is detected in mucopolysaccharidosis (MPS) type VI (Maroteaux-Lamy syndrome)