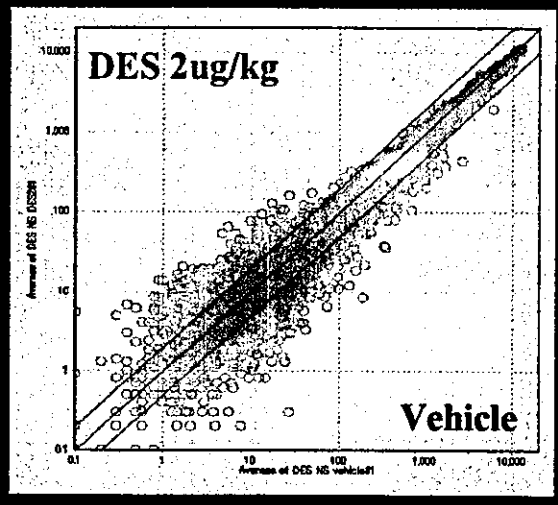
GeneChip analysis

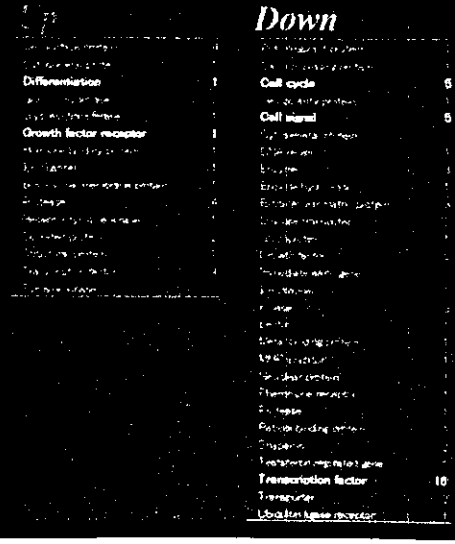


MGU74v2
(Affymetrix GeneChip)
Mouse. 12,000 genes

DES changed gene expression to differentiation preferable state

	Total	*Known*	*EST*
Up	56	27	29
Down	129	67	62

Category of known genes



Differentiationrelated gene
PDGF receptor

EGF receptor
Cell cycle
Cell signal
Transcription factor
(c-fos etc.)

Quantitative RT-PCR condition

1. Sybergreen system
2. Normalize to total RNA quantity monitored with spiked PHE RNA quantity

1	M24537	PHE
2	M80540	DLX-2
3	X04367	PDGFR
4	M32490	IGFBP10
5	X64713	CycB1
6	AW049716	EGFR
7	U20735	JUNB
8	M95200	VEGF
9	X81580	IGFBP2
10	M28845	KROX-24
11	V00727	c-FOS

Black=SPIKE
Red=UP
Blue=DOWN

- 恒常性維持機構研究

  エピジェネティック制御機構障害の神経幹細胞をモデルにした研究

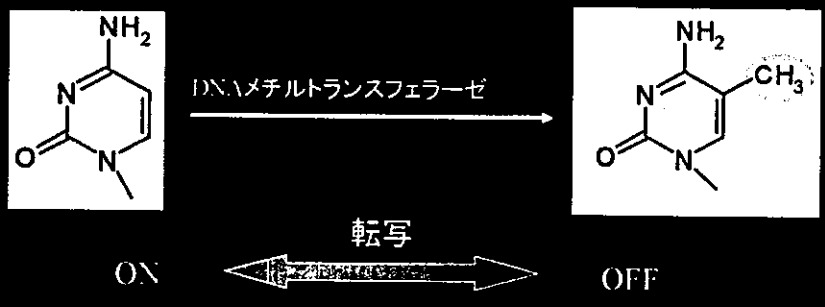
エピジェネティクス

- DNAの配列変化を伴わずに子孫や娘細胞に伝達される遺伝子機能の変化

  DNAメチル化／脱メチル化

  ヒストン修飾（クロマチン構造変換）

DNAメチル化



DNAメチル化による制御

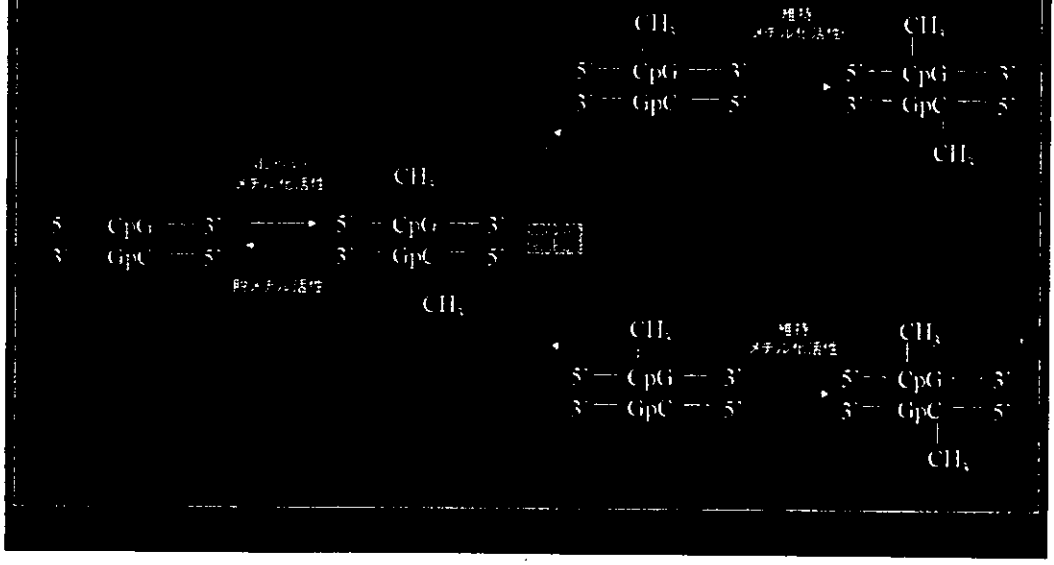
- 発生
  - Reprogramming
    (世代ごとにエピジェネティック
    制御がリセット)
  - X染色体不活性化
  - 発生・分化調節:
    - CNS形成・分化過程における恒常性維持
      機構のひとつ
- 疾患
  - がん等

エピジェネティック作用を有するとされる
化合物例

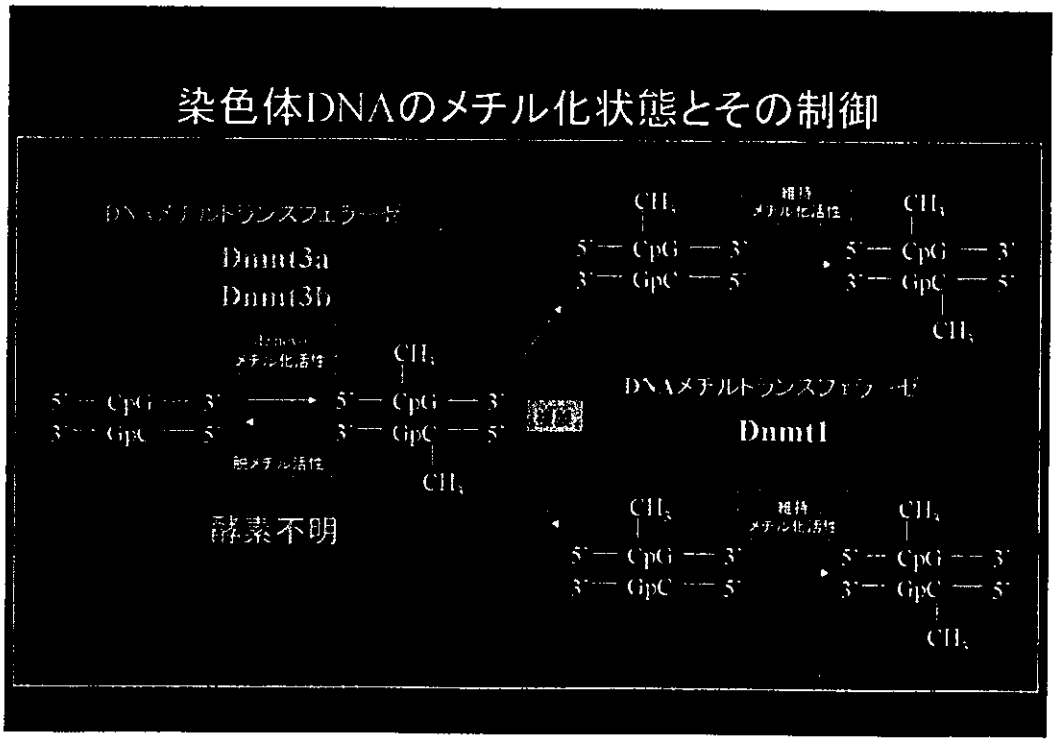
化学物質名	作用		
	メチル化促進	脱メチル化促進	アセチル化促進
5-アザシチジン	×	○	×
ヒ素	○	○	×
ニッケル	○	×	×
Procainamide	×	○	×
Procaine	×	○	×
Valproic acid	×	×	○
Trichostatin A	×	×	○

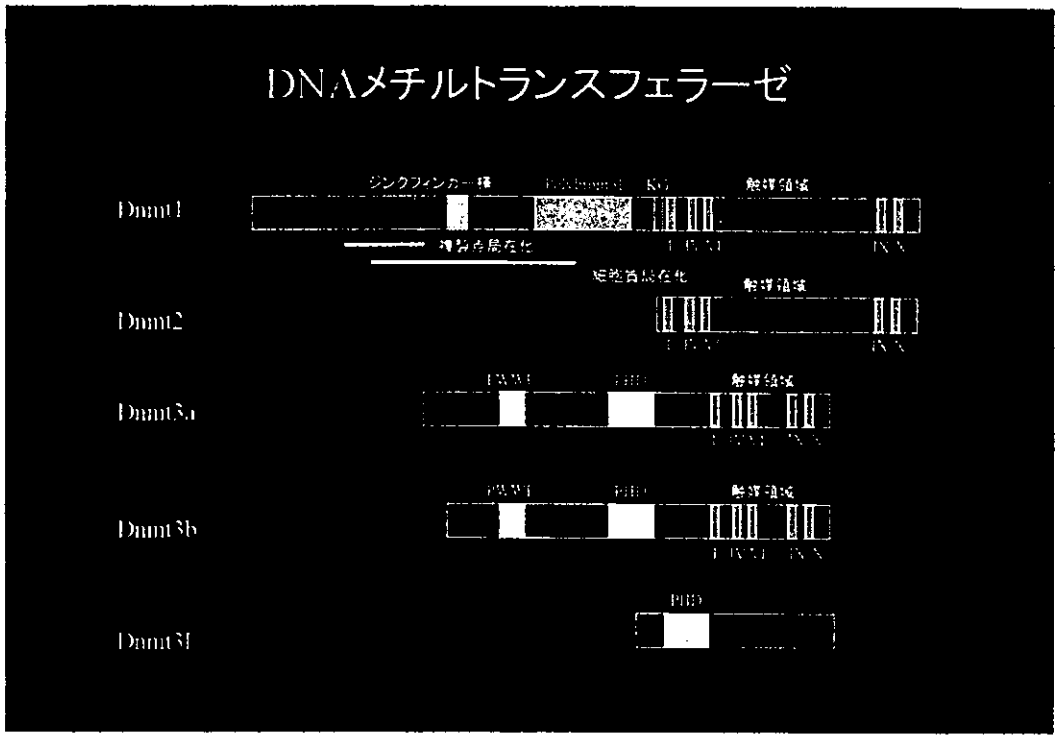
こんなに少ないのか？

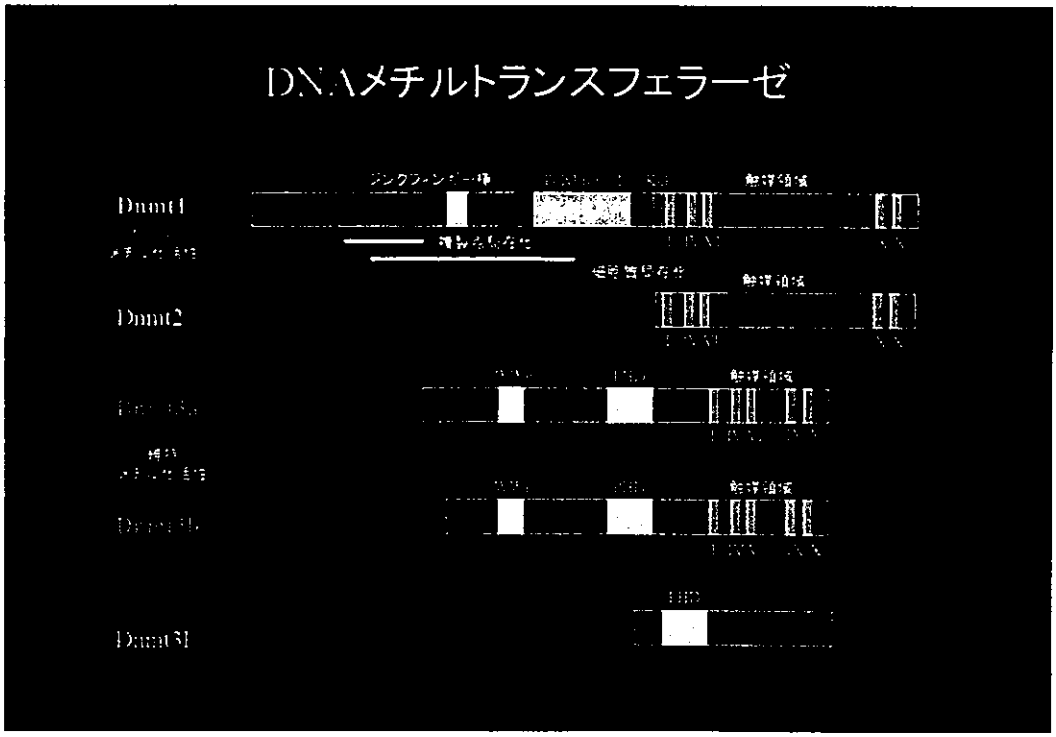
染色体DNAのメチル化状態とその制御

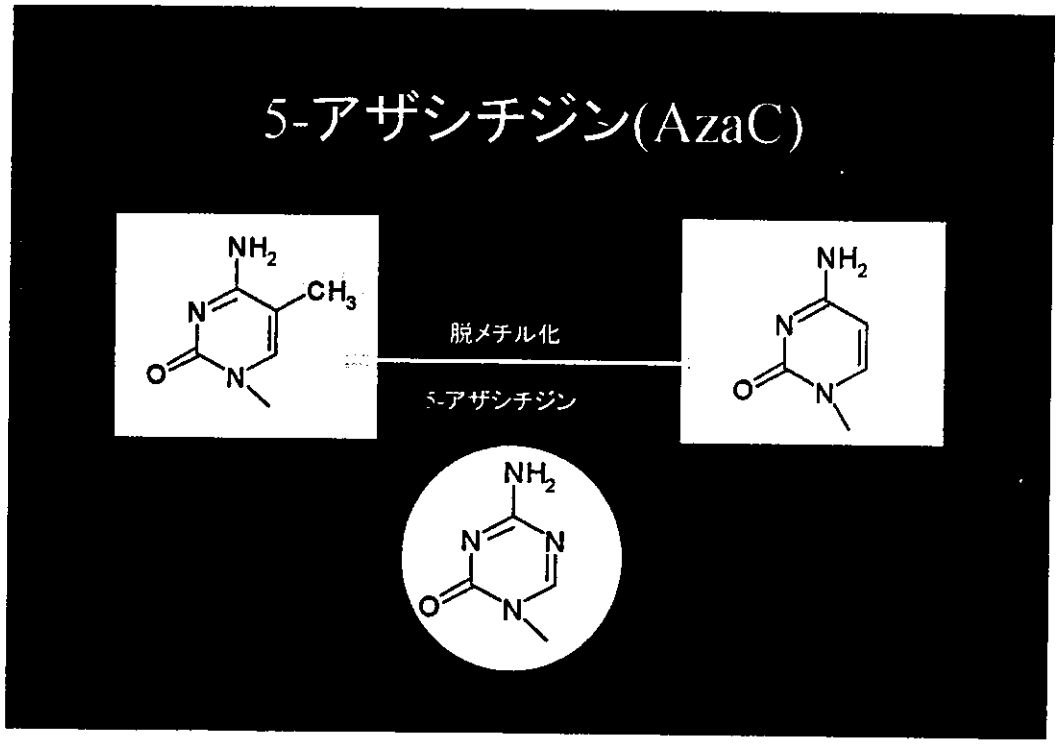


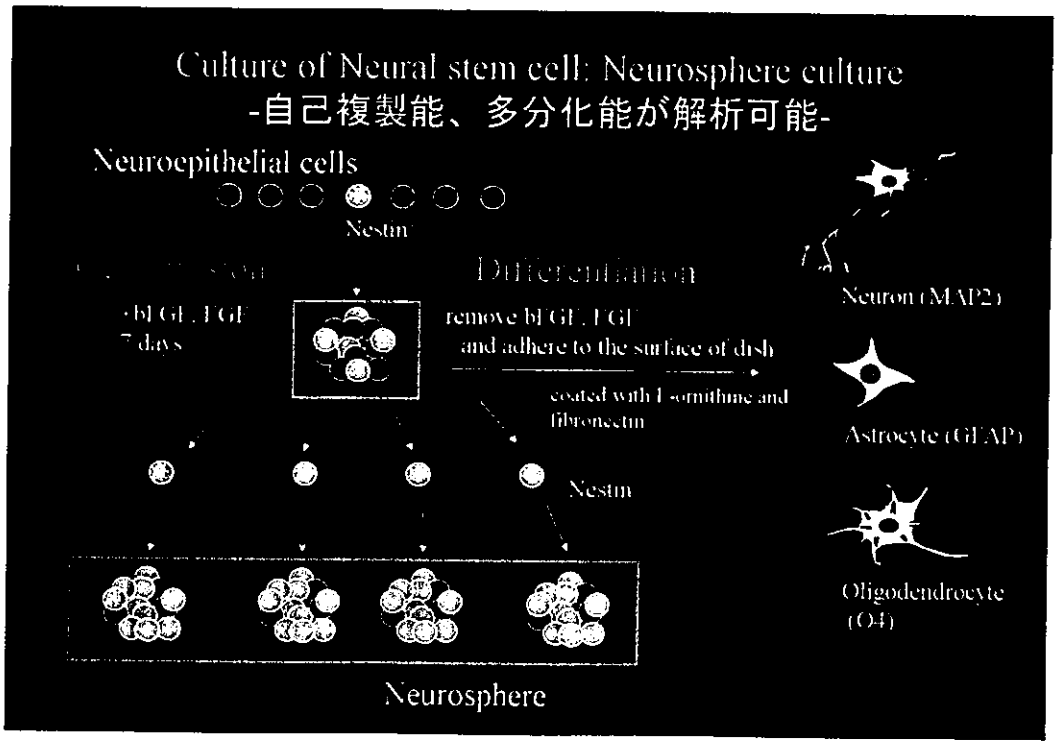
染色体DNAのメチル化状態とその制御



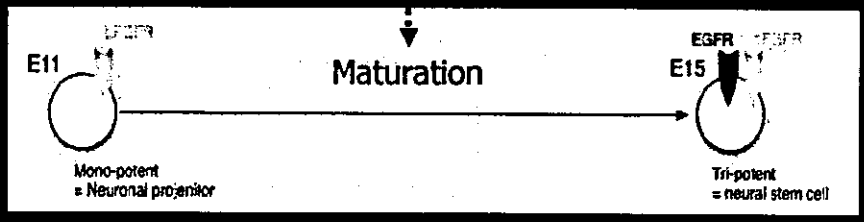

DNAメチルトランスフェラーゼ
Dnmt1
Dnmt2
Dnmt3a
Dnmt3b
Dnmt3L


DNAメチルトランスフェラーゼ
Dnmt1
Dnmt2
Dnmt3L


5-アザシチジン(AzaC)
脱メチル化
5-アザシチジン


Culture of Neural stem cell: Neurosphere culture
-自己複製能、多分化能が解析可能-
Neuroepithelial cells
Nestin
Differentiation
remove bFGF, EGF
and adhere to the surface of dish
coated with L-ornithine and fibronectin
Nestin
Neurosphere
Neuron (MAP2)
Astrocyte (GFAP)
Oligodendrocyte (O4)

1. NSC の「成熟」の概念とその過程への影響の解析
2. Stem cell の　自己複製と分化への影響の解析

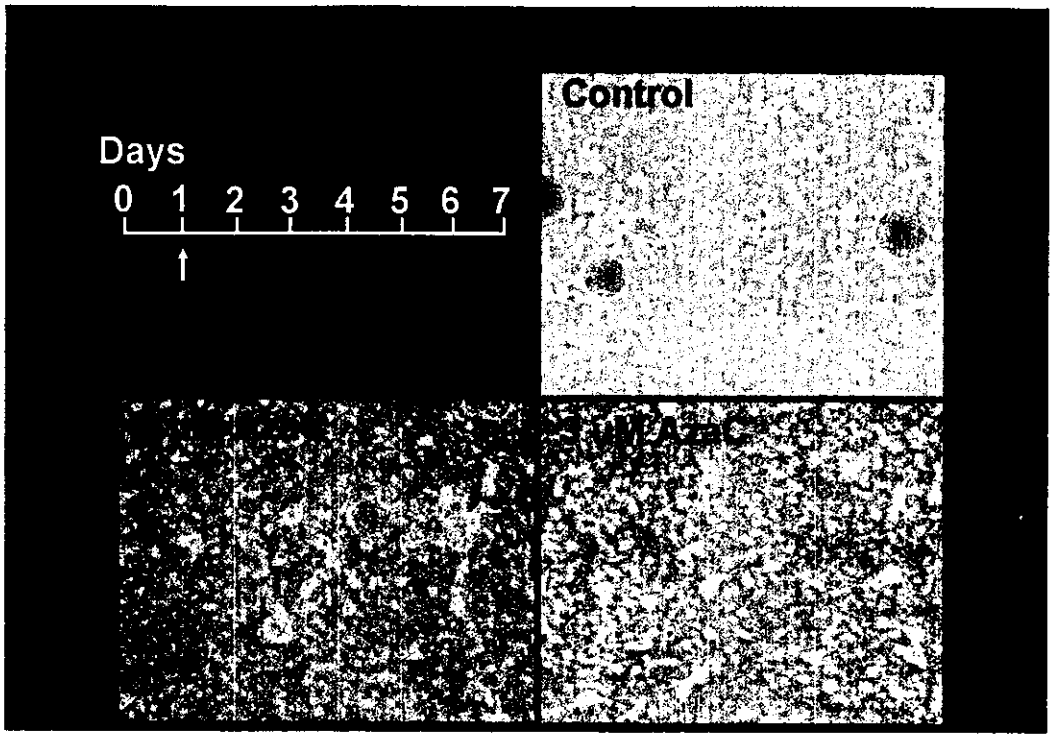


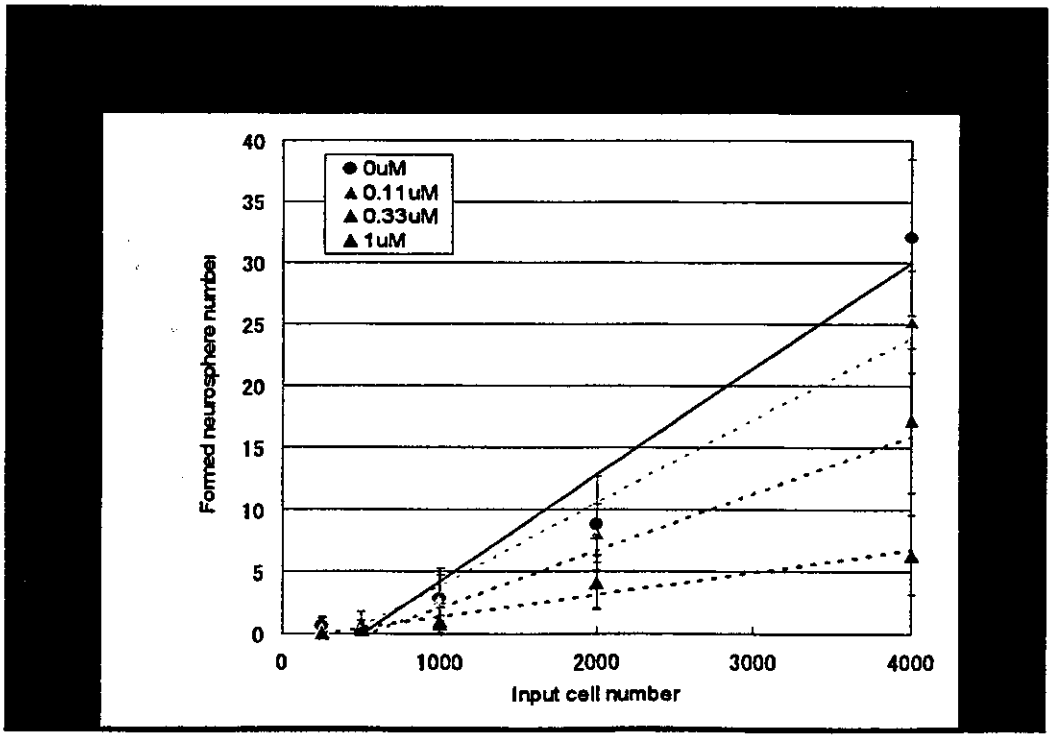
研究方法

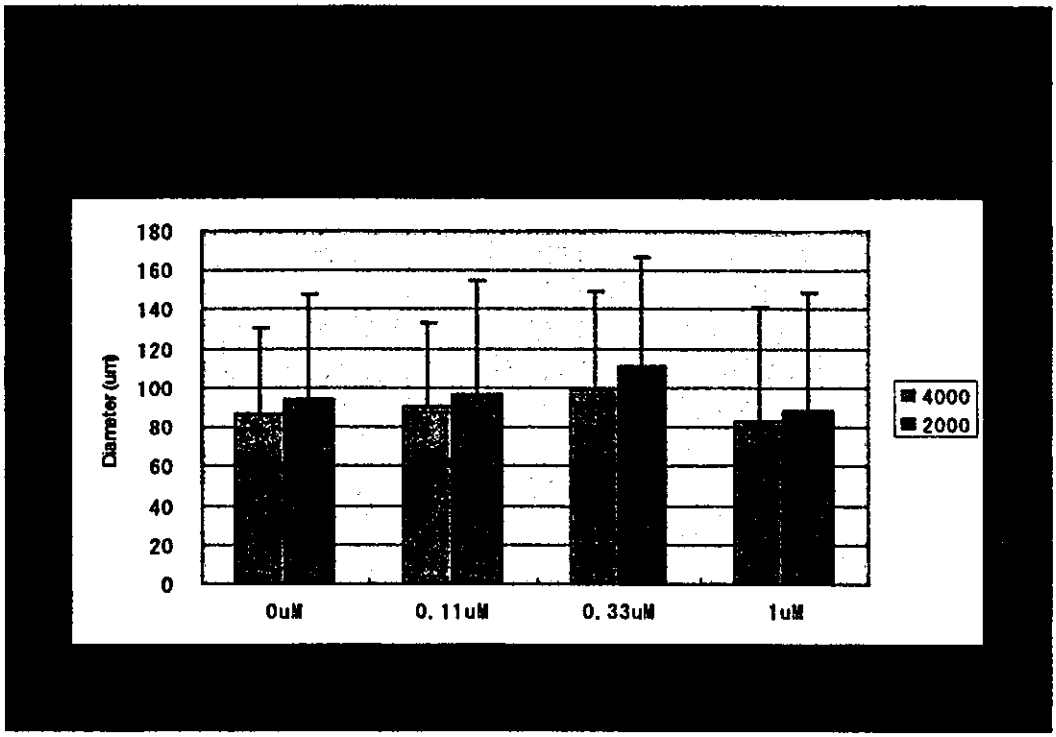
胎生14.5日のマウス胎児の終脳から神経上皮細胞を分離

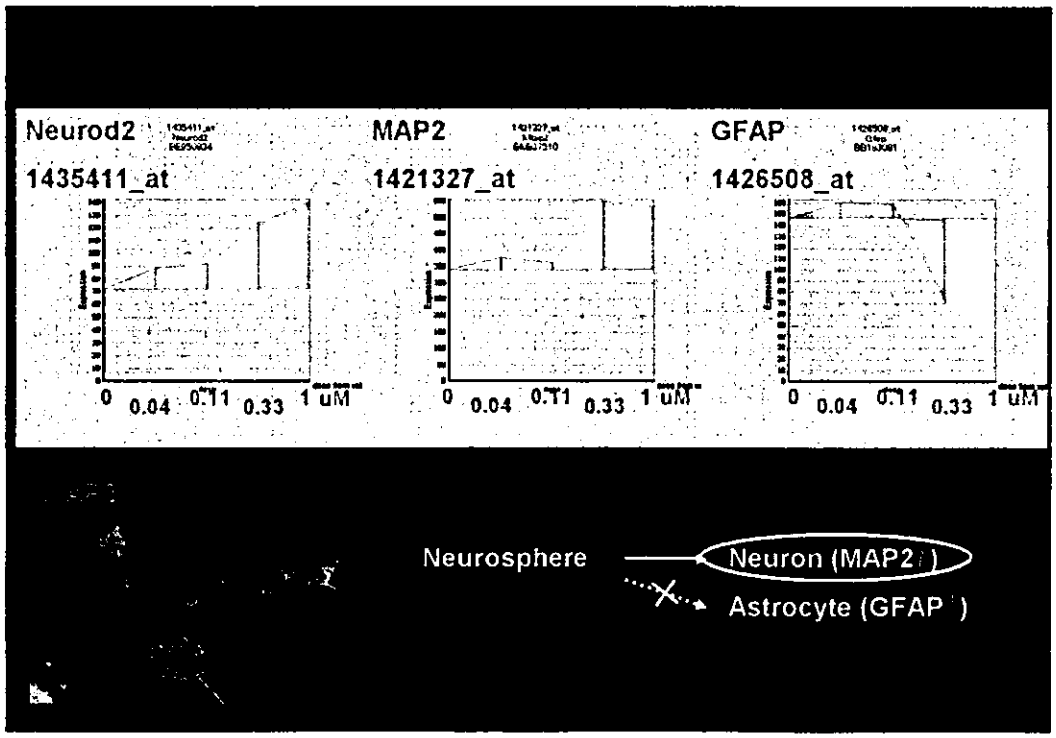
分離した神経上皮細胞をDNAの脱メチル化剤であるAzaCで処理し，浮遊細胞凝集塊（ニューロスフェアー）を形成させた.

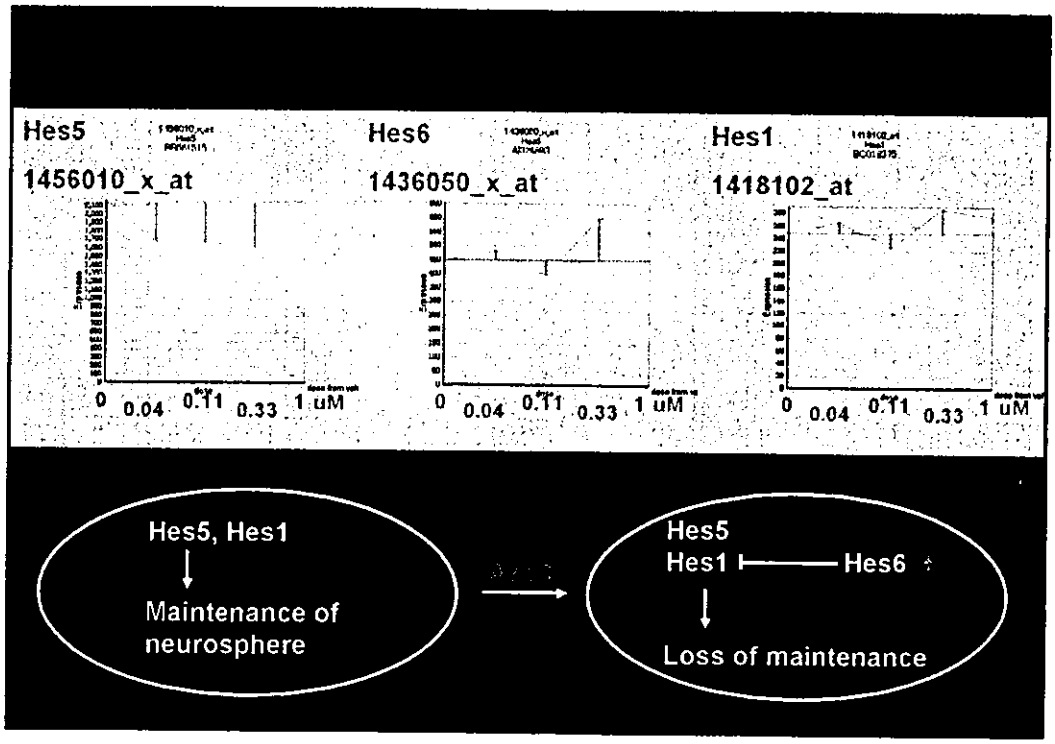
形態観察に加え，GeneChip解析(Percellome手法)により，遺伝子発現の変化を解析した.


Days
0 1 2 3 4 5 6 7
Control
1μM AzaC


0uM
0.11uM
0.33uM
1uM
Formed neurosphere number
Input cell number
0
5
10
15
20
25
30
35
40
0
1000
2000
3000
4000

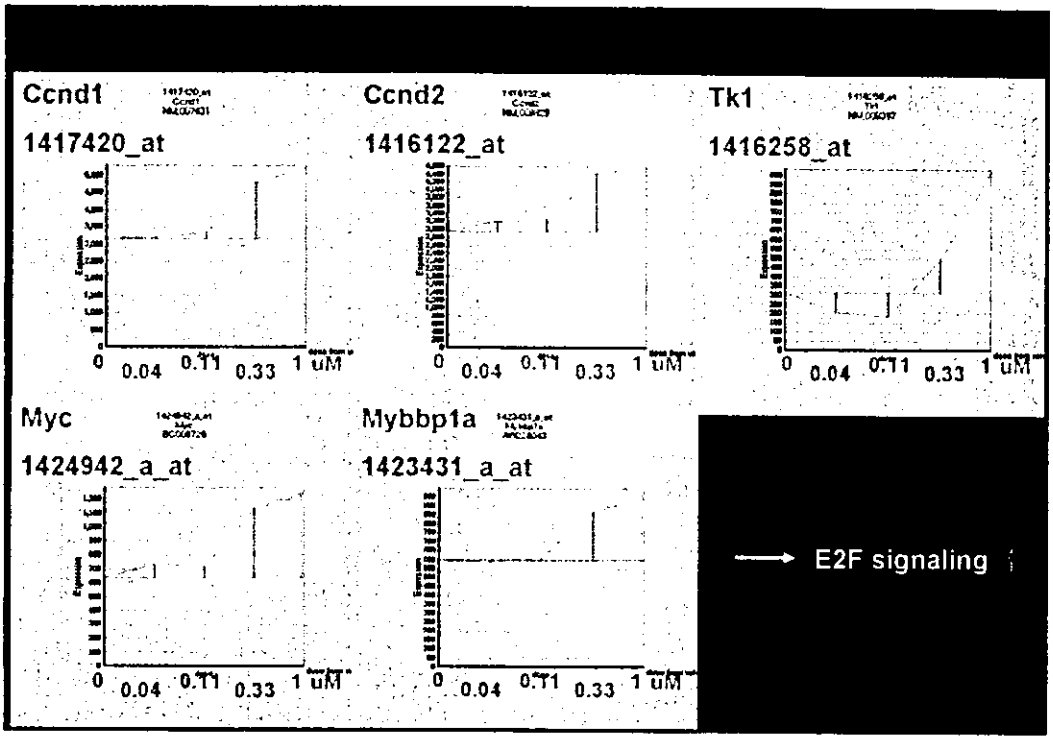

180
160
140
120
100
80
60
40
20
0
Diameter (um)
0uM
0. 11uM
0. 33uM
1uM
4000
2000

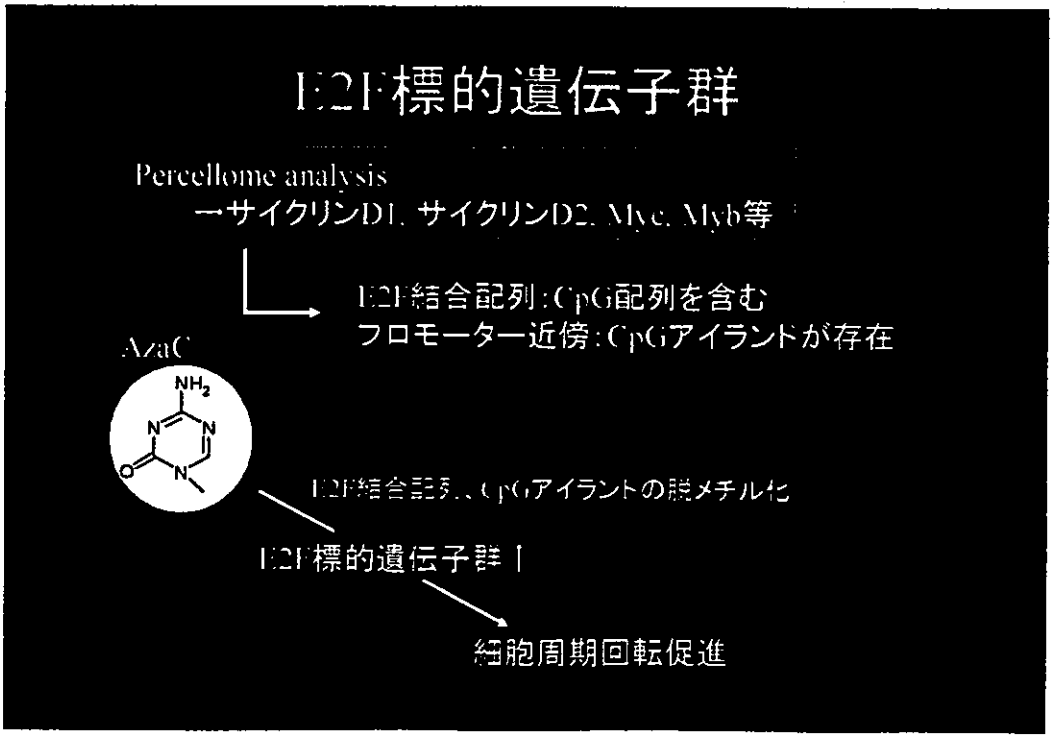

Neurod2
1435411_at
MAP2
1421327_at
GFAP
1426508_at
0
0.04
0.11
0.33
1 uM
Neurosphere
Neuron (MAP2)
Astrocyte (GFAP)

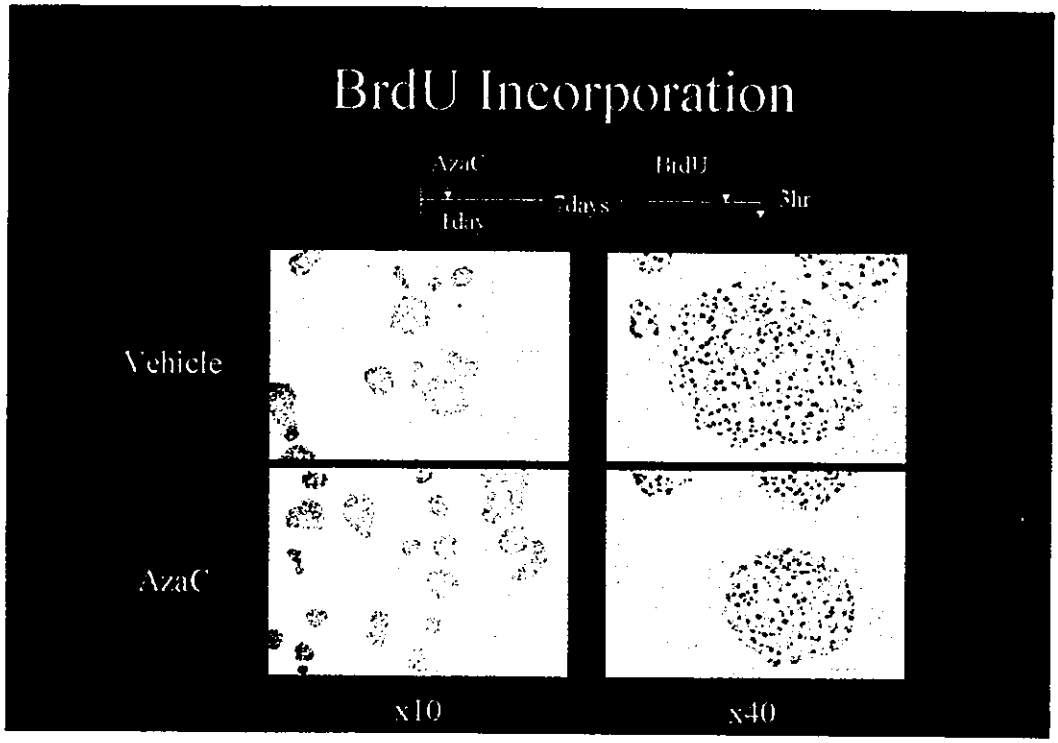


AzaCにより，ニューロスフェアーの形成数は減少したが，形成されたニューロスフェアーの大きさには違いは認められなかった．

AzaCは，非特異的にゲノムを脱メチル化しているために，他のシグナルが影響を受けている可能性がある．


Ccnd1
1417420_at
Ccnd2
1416122_at
Tk1
1416258_at
Myc
1424942_a_at
Mybbp1a
1423431_a_at
0 0.04 0.11 0.33 1 uM
E2F signaling


E2F標的遺伝子群
Percellome analysis
ーサイクリンD1, サイクリンD2, Myc, Myb等
E2F結合配列:CpG配列を含む
フロモーター近傍:CpGアイランドが存在
AzaC
NH2
E2F結合配列、CpGアイランドの脱メチル化
E2F標的遺伝子群↑
細胞周期回転促進



AzaCの神経幹細胞に対する作用

形態：

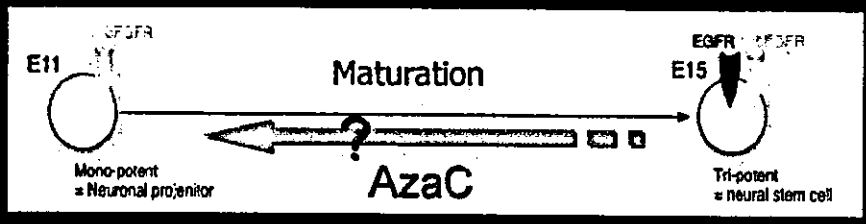
・ニューロン分化促進
（神経突起伸長、MAP2陽性）

・NS直径不変

遺伝子発現：

・ニューロン分化促進
（MAP2↑，Notch系↓）

・E2F標的遺伝子発現上昇による
細胞周期回転促進



Neuron分化傾向
増殖促進傾向

エピジェネティック作用を有するとされる
化合物例

化学物質名	作用		
	メチル化促進	脱メチル化促進	アセチル化促進
5-アザシチジン	×	○	×
ヒ素	○	○	×
ニッケル	○	×	×
Procainamide	×	○	×
Procaine	×	○	×
Valproic acid	×	×	○
Trichostatin A	×	×	○

考　察

- 化学物質によるエピジェネティック制御機構障害は、[redacted]を介し、神経幹細胞のいわゆる「成熟過程」を逆行させることで、個体の恒常性維持機構の破綻を来たす可能性が示唆される。

- DNAメチル化修飾機構の有無の[redacted]の開発が期待される。

化学物質影響の網羅的遺伝子発現解析データベース化

END

別添５

研究成果の刊行に関する一覧表レイアウト

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
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雑誌

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