

製を重度に抑制あるいは停止させた後、G2期に入る前に apoptosis を誘発し、また、5AzC は DNA 複製中の神経上皮細胞に取り込まれ M 期で細胞周期を停滞させた後、分裂後の G1 期に apoptosis を誘発した。

F. 健康危機管理情報  
なし

G. 研究発表

1. 論文発表

Katayama K, Ueno M, Takai H, Ejiri N, Uetsuka K, Nakayama H, Doi K, Ethylnitrosourea induces apoptosis and cell cycle arrest in the trophoblastic cells of the rat placenta, Biol.Reprod., 67,431-435, 2002.

Ueno M, Katayama K, Nakayama H, Doi K, Mechanisms of 5-azacytidine (5-AzC)-induced toxicity in the rat foetal brain Int.J.Exp.Pathol., 83,139-150, 2002.

Ueno M, Nakayama H, Kajikawa S, Katayama K, Suzuki K, Doi K, Expression of ribosomal protein L4 (rpL4) during neurogenesis and 5-azacytidine (5AzC)-induced apoptotic process in the rat, Histol.Histopathol., 17, 789-798, 2002.

Katayama K, Ohtsuka R, Takai H, Nakayama H, Doi K, Expression of p53 and its transcriptional target genes mRNA in the ethylnitrosourea-induced apoptosis and cell cycle arrest in the fetal central nervous system, Histol.Histopathol., 17,715-720,2002.

Ejiri N, Katayama K, Yakayama H, Doi K, Expression of cytochrome P450 (CYP) isozymes in rat placenta through pregnancy, Exp.Toxicol.Pathol., 53,387-391,2001.

Katayama K, Uetsuka K, Ishigami N, Nakayama H, Doi K, Apoptotic cell death and cell proliferative activity in the rat fetal central nervous system from dams administered with ethylnitrosourea (ENU), Histol.Histopathol., 16,79-85,2001.

Ishigami N, Shinozuka J, Nakayama H, Doi K, Apoptosis in mouse fetuses from dams exposed to T-2 toxin at different days of gestation, Exp.Toxicol.Pathol., 52, 493-501, 2001.

H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

**T-2 Toxinの妊娠ラットにおける遺伝子発現解析－  
GeneChip解析－**

試験デザイン

動物： Slc:Wistarラット(妊娠13日)  
 被検物質： T-2 toxin (Sigma)  
 投与用量： 0 (Corn oil), 2 mg/kg  
 投与経路： p.o.  
 解剖： 投与24時間後  
 動物数： 3例/群＝計6例  
 検査項目： 一般状態  
             体重, 器官重量  
             胎仔数, 胎仔体重  
             病理組織学的検査

GeneChip解析(Chip: Affymetrix Rat Genome U34A)

親肝臓  
 胎仔肝臓  
 胎盤

病理組織学的所見－親

Organs	Histopathological findings	Time after treatment (hr)	
		0	24
		Dose (mg/kg)	
		No. of animals	
Liver	Single cell necrosis of hepatocyte	3	3
	Focal necrosis of hepatocyte	0	1
	Microvesicular steatosis	0	2
	Cell infiltration	0	3
Thymus	Atrophy of cortex	0	3
	Lymphoid necrosis	0	3
Spleen	Lymphoid necrosis at white pulp	0	3
	Decrease in hematopoiesis	0	0
Fore stomach	Erosion	0	3
	Edema and cell infiltration in submucosa	0	1
	Hyperplasia of squamous cell	0	0
Glandular stomach	Hemorrhage in mucosa	0	2
	Necrosis of epithelium	0	2
	Edema and cell infiltration in submucosa	0	1
Jejunum/Ileum	Single cell necrosis of epithelium at crypt	0	1
Uterus	Hemorrhage at placenta	0	1
Placenta	Hemorrhage	0	1
	Single cell necrosis of cytotrophoblast	0	1

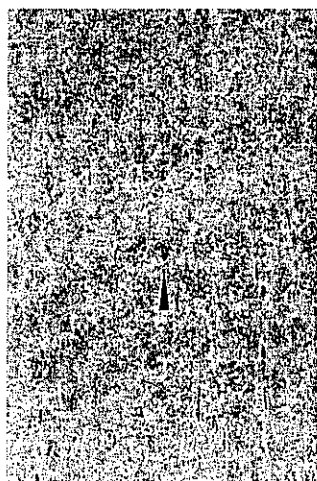
病理組織学的所見—胎仔

Organ	Pathological findings	Time after dosing (hr)	24	
		Dose (mg/kg)	No. of animals	No. of fetuses examined
Liver	Single cell necrosis of hepatocyte	0	3	13
	Single cell necrosis of hematopoietic cell	-	3	12
Nervous system	Single cell necrosis in telencephalon	±	±	±
	Single cell necrosis in diencephalon	±	±	±
	Single cell necrosis in mesencephalon	±	±	±
	Single cell necrosis in metencephalon	±	±	±
	Single cell necrosis in myelencephalon	±	±	±
	Single cell necrosis in spinal cord	±	±	±
	Single cell necrosis in ganglion	±	±	±
	Single cell necrosis in olfactory epithelium	±	±	±
Tongue, laryngeal-mesenchyma	Single cell necrosis	±	±	±
Cartilage primordium	Single cell necrosis	±	±	±
Mesonephric tissue	Single cell necrosis	±	±	±
Intestine	Single cell necrosis	±	±	±

Note: -, Almost absent; ±, Very slight; +, Slight; ++, Moderate.

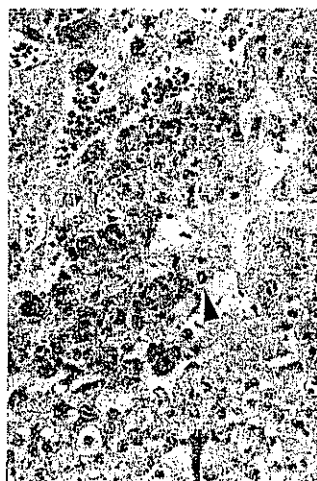
Photographs of Histopathological findings

Dam Liver



Fatty change and single cell necrosis of hepatocyte

Placenta



Single cell necrosis of cytotrophoblasts

Fetus Liver



Single cell necrosis of hematopoietic cells and hepatocytes

## GeneChip解析の流れ

Total RNA抽出

cDNA合成

cRNA合成, 精製, 断片化

ハイブリダーゼーション

イメージ取得

データ解析

- ① Trimmed mean (4%) normalization
- ② Distinction analysis (解析ソフト: Spotfire)  
群Aの平均と群Bの平均の差を、群Aと群Bの分散の分散の和で  
除した値  
$$\text{Distinction value} = (\text{AverageA} - \text{AverageB}) / (\text{stdA} + \text{stdB})$$
- ③ EST除去
- ④ Absenceプローブ除去  
(発現量増加群では増加群で全てPresenceのものを選択)
- ⑤ Distinction value (+1.5 or -1.5) に関してデータをsorting

## Summary of extracted probes

T2-toxin

Trimmed mean normalization (4%)

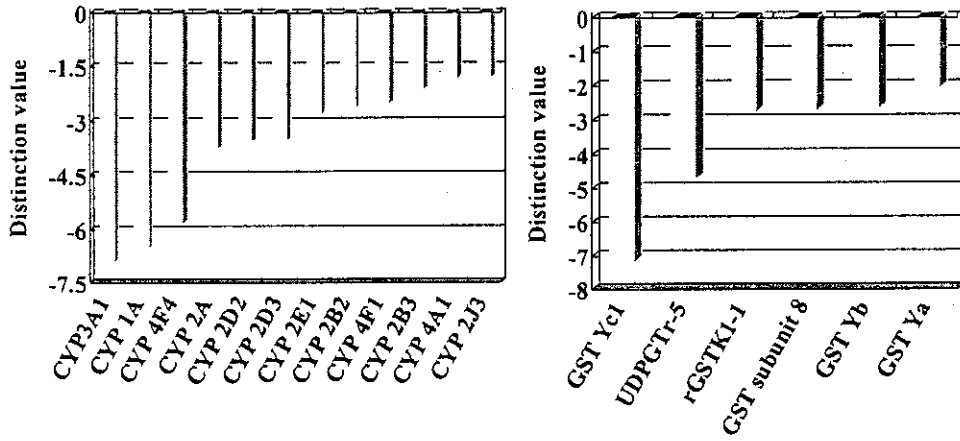
Dam liver		Fetus liver	
increase	decrease	increase	decrease
235	382	15	103

Placenta	
increase	decrease
187	181

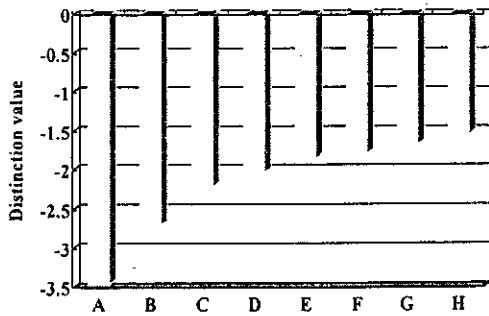
— 親肝臓 —

薬物代謝関連遺伝子



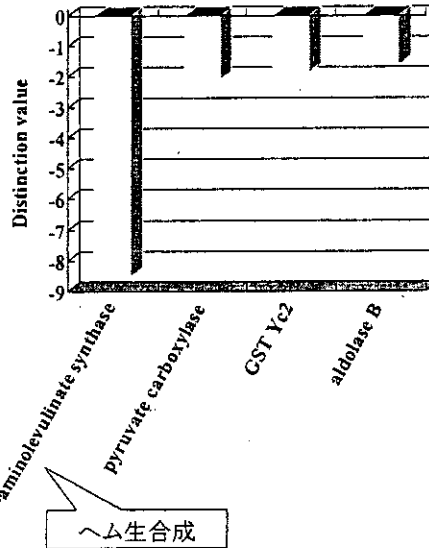
— 胎仔肝臓 —

脂質代謝関連遺伝子



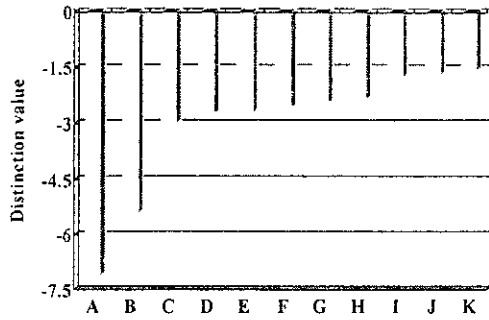
- A: squalene epoxidase
- B: HMG-CoA synthase
- C: apolipoprotein B
- D: fatty acid transporter
- E: mevalonate pyrophosphate decarboxylase
- F: acyl-CoA synthetase 5
- G: acyl coenzyme A dehydrogenase medium chain
- H: very-long-chain Acyl-CoA dehydrogenase

その他代謝関連遺伝子

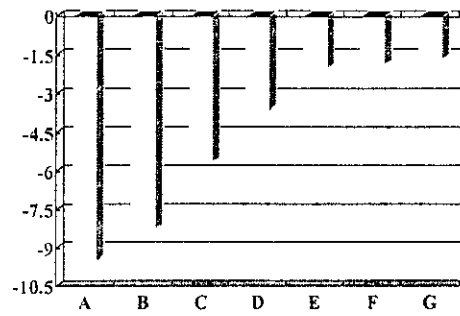


— 親肝臓 —

脂質代謝関連遺伝子



ミトコンドリア関連遺伝子

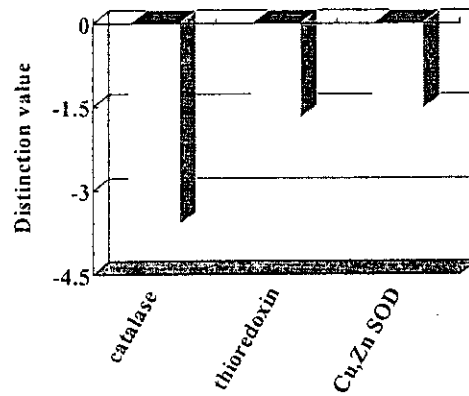
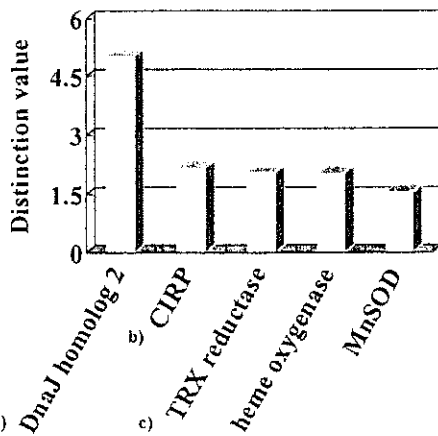


- A: enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase  
 B: very-long-chain acyl-CoA synthetase  
 C: acyl-CoA synthetase 5  
 D: fatty acid synthase  
 E: mitochondrial acetoacetyl-CoA thiolase precursor  
 F: apolipoprotein A-I (apoA-I)  
 G: short chain acyl-coenzyme A dehydrogenase  
 H: apolipoprotein E  
 I: HMG-CoA synthase  
 J: peroxisomal 3-ketoacyl-CoA thiolase  
 K: apolipoprotein C-III

- A: ornithine carbamoyltransferase  
 B: soluble cytochrome b5  
 C: NADH-cytochrome b-5 reductase  
 D: ATP synthase subunit c  
 E: mitochondrial succinyl-CoA synthetase alpha subunit  
 F: cytochrome c oxidase subunit VIII  
 G: mitochondrial NADH dehydrogenase

— 親肝臓 —

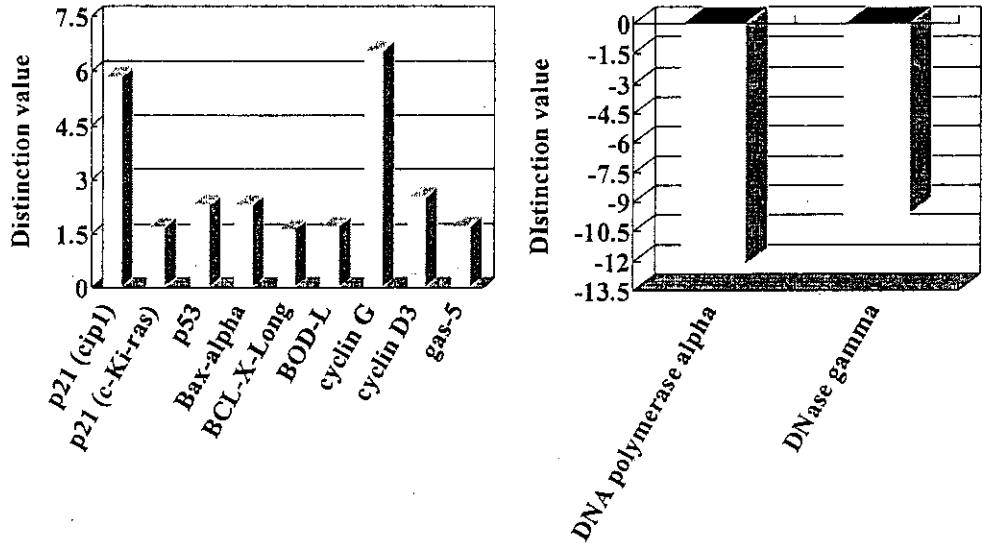
酸化ストレス関連遺伝子



- a) cochaperone of HSP70  
 b) cold-inducible RNA-binding protein  
 c) thioredoxin reductase

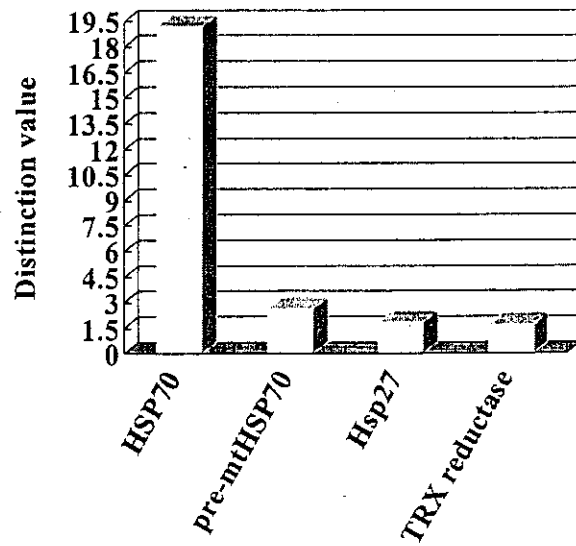
— 親肝臓 —

細胞周期関連遺伝子



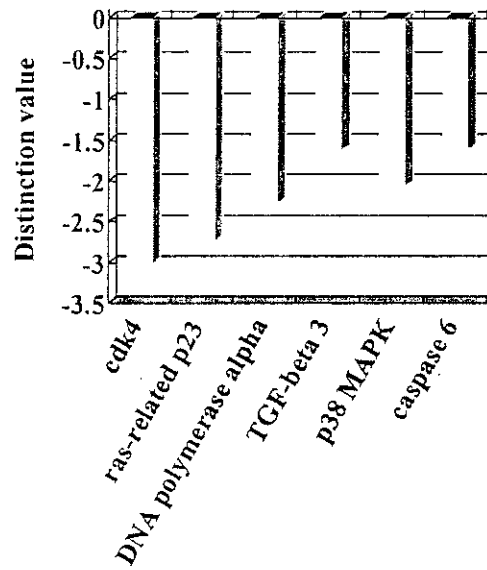
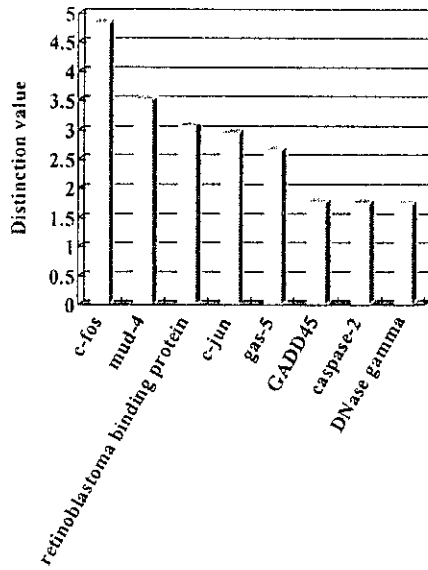
— 胎盤 —

酸化ストレス関連遺伝子



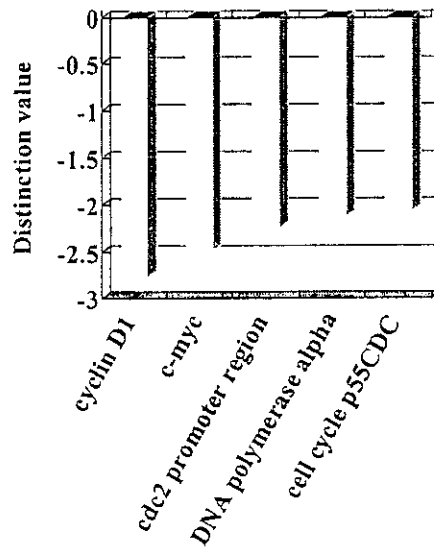
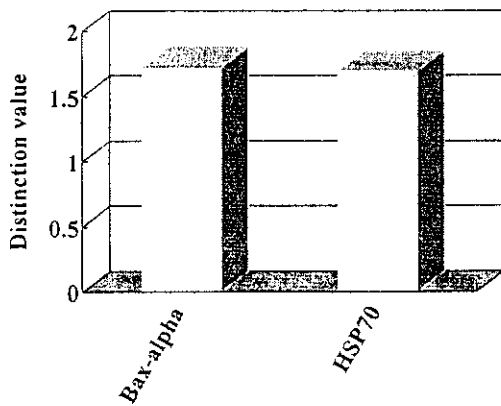
— 胎盤 —

細胞周期関連遺伝子等



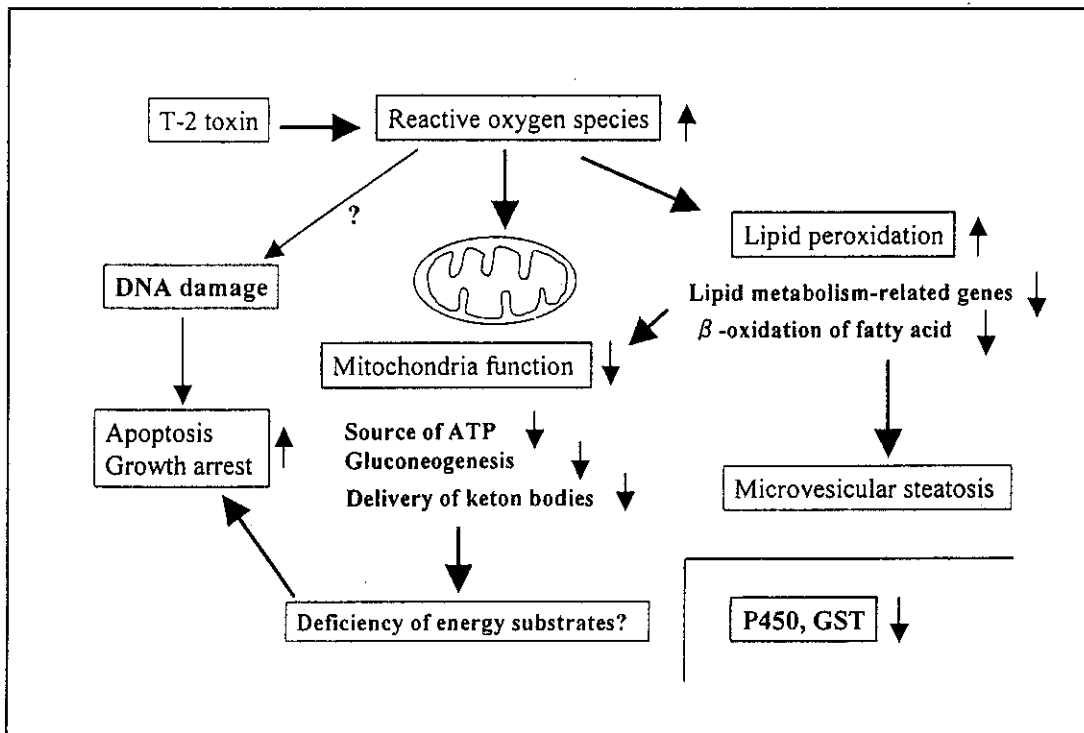
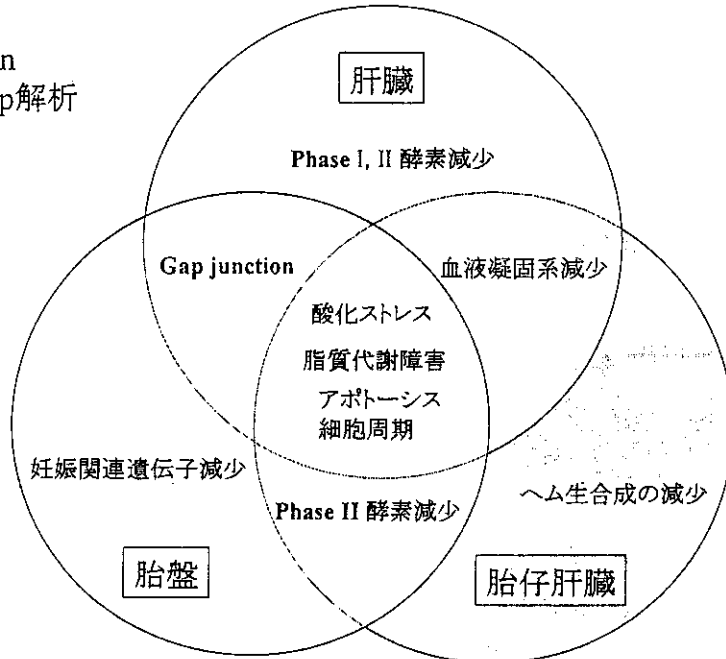
— 胎仔肝臓 —

細胞周期・酸化ストレス関連遺伝子



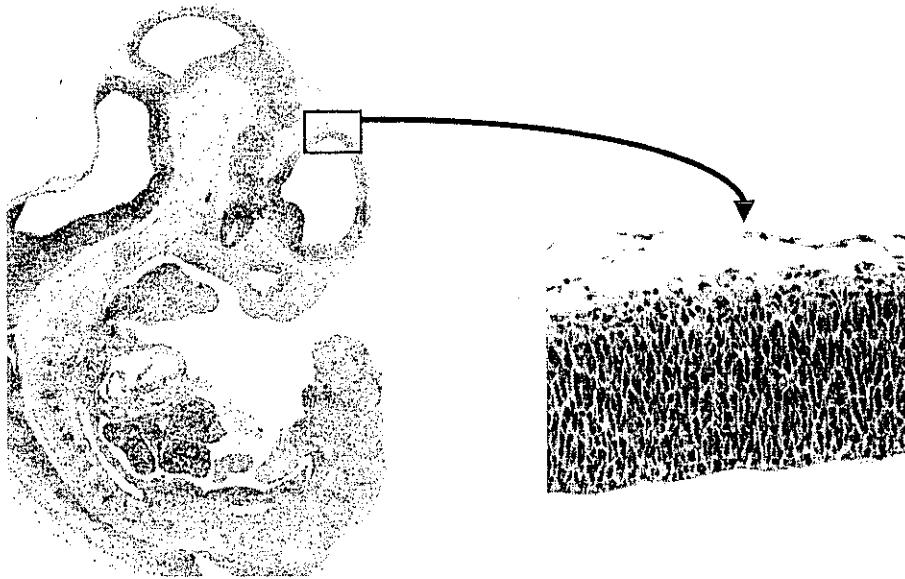


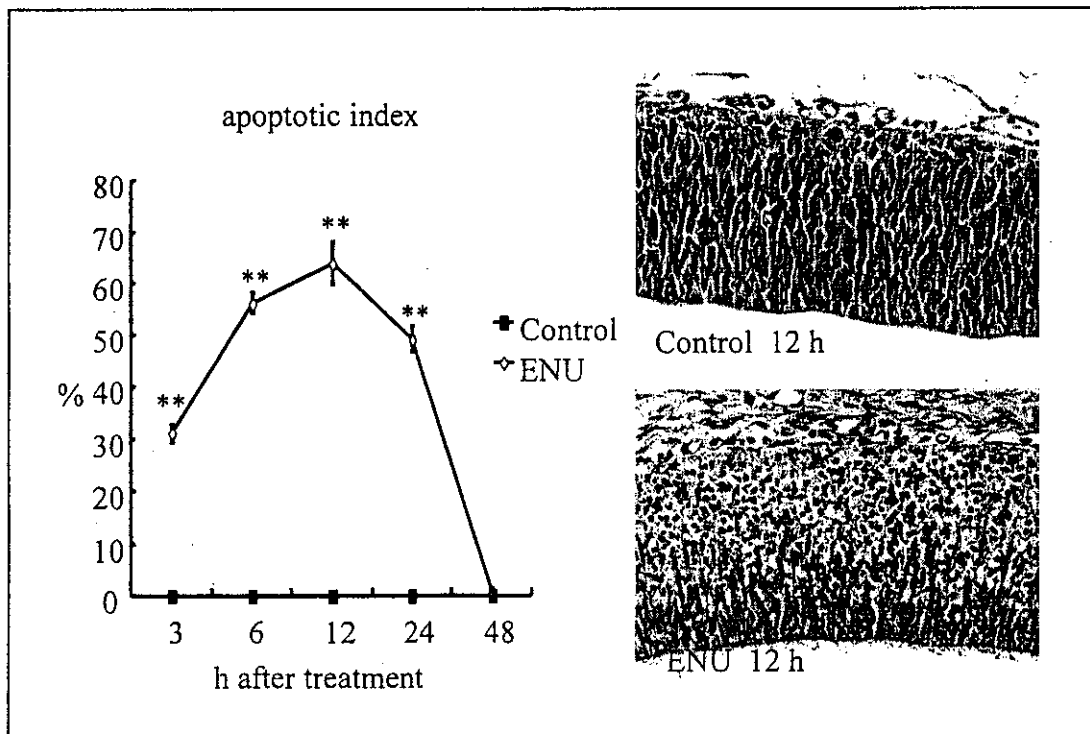
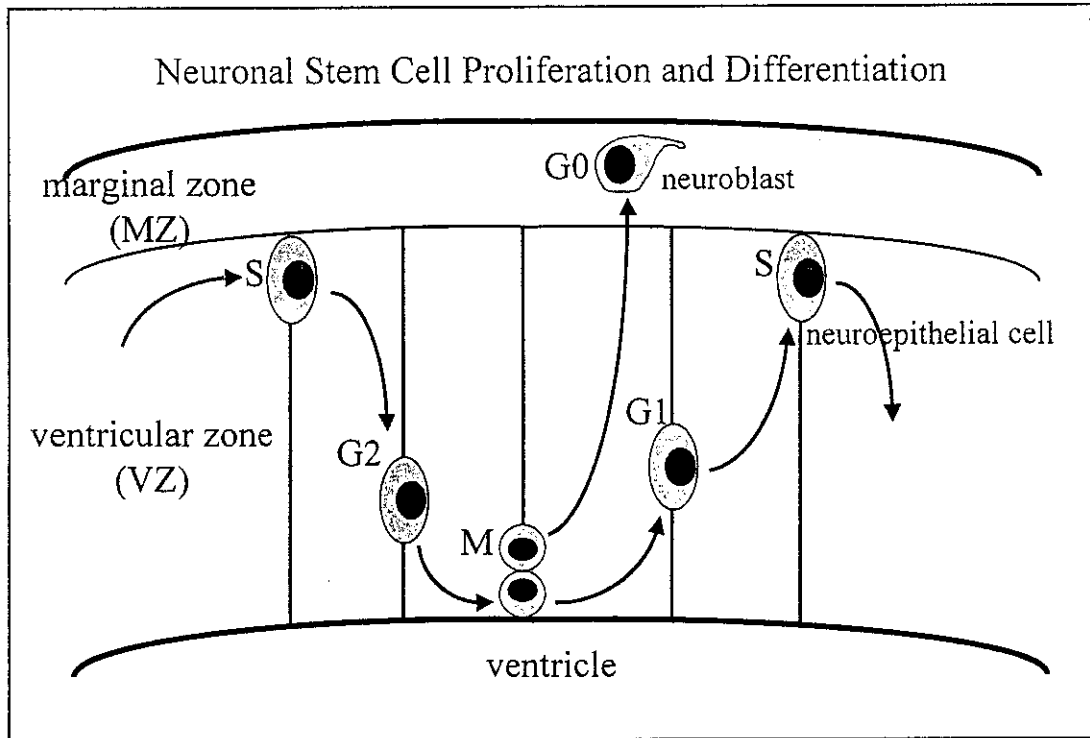
T-2 Toxin  
GeneChip解析  
まとめ

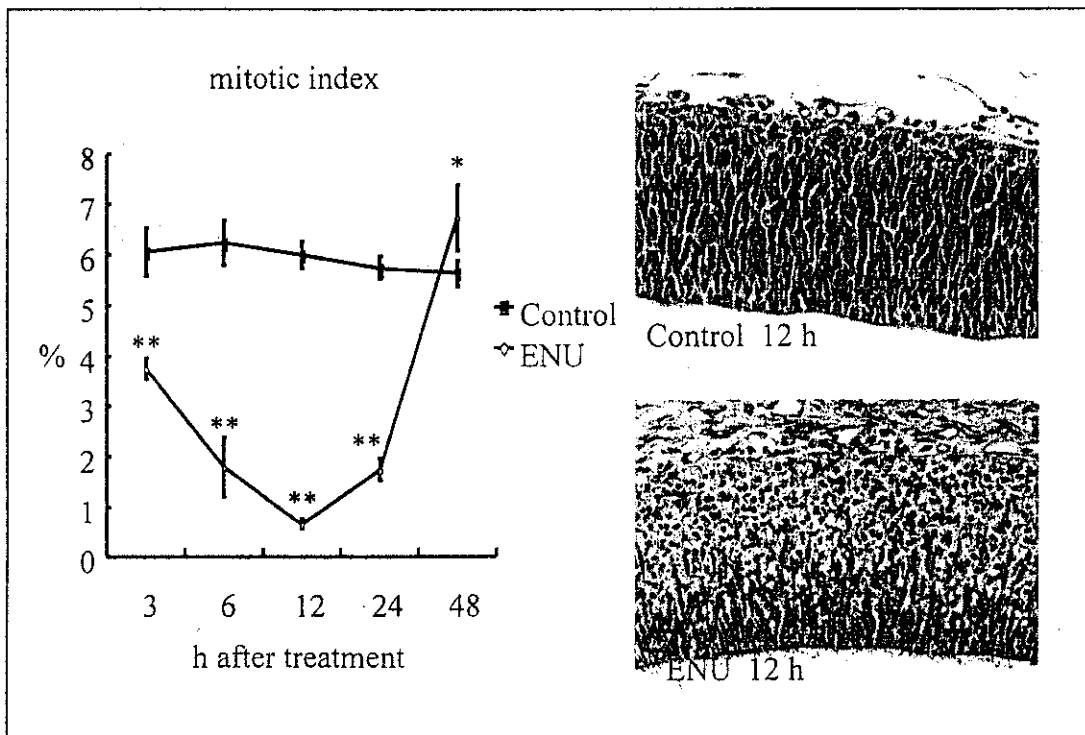
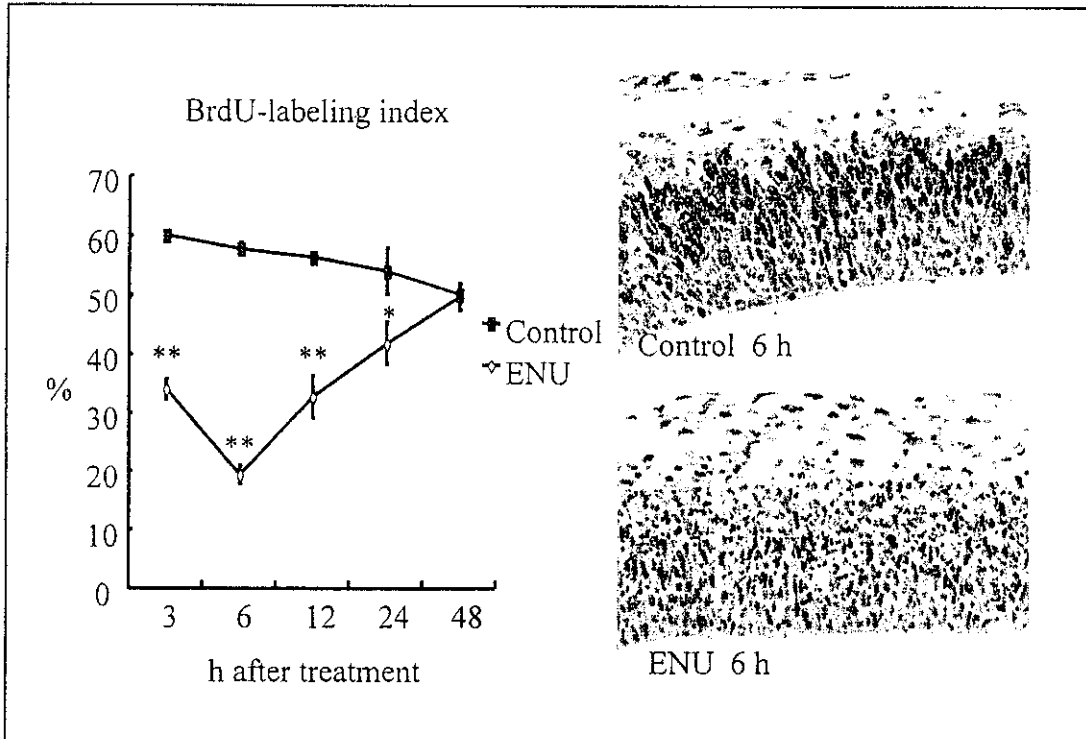


Mechanism of ethylnitrosourea-induced fetal  
central nervous injury  
—— cell cycle arrest and apoptosis ——

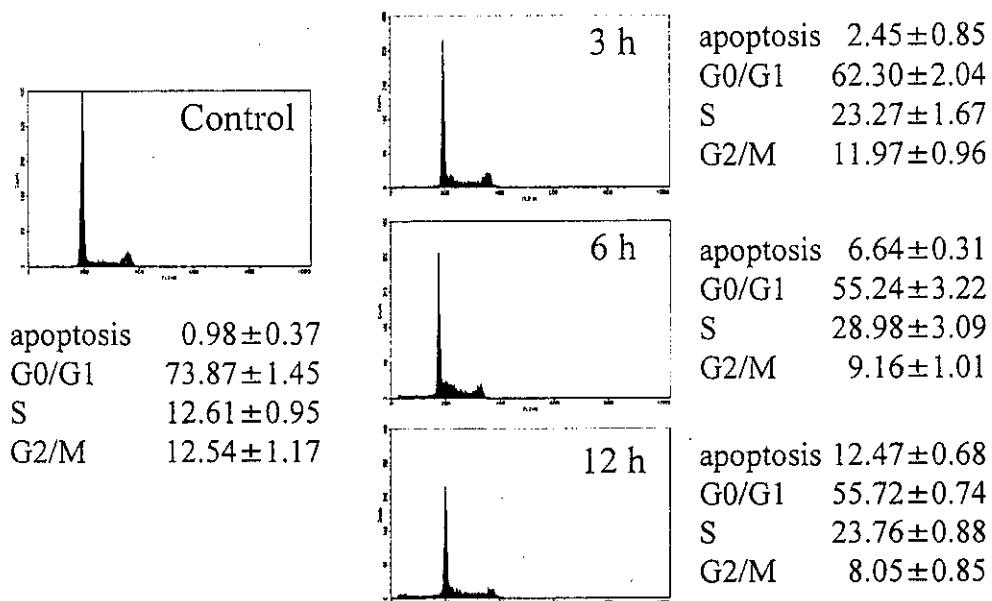
Neuronal Stem Cell Proliferation and Differentiation



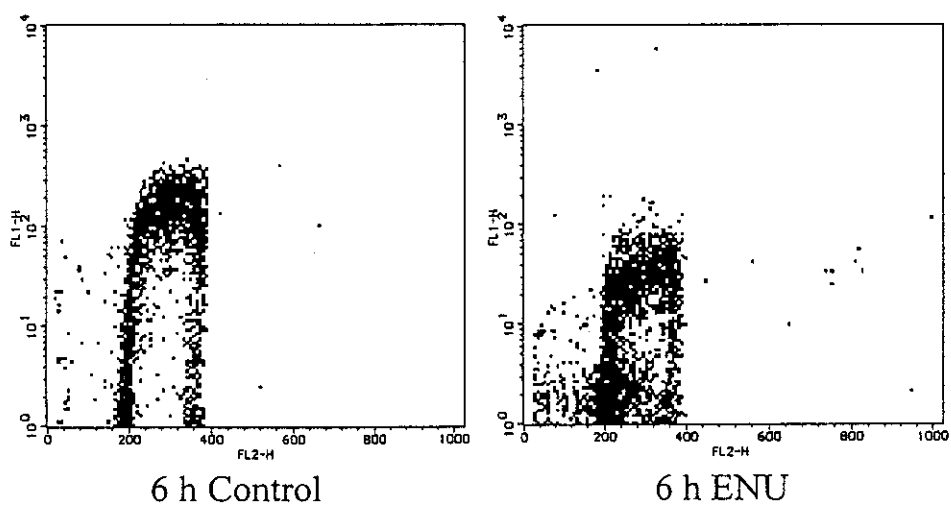


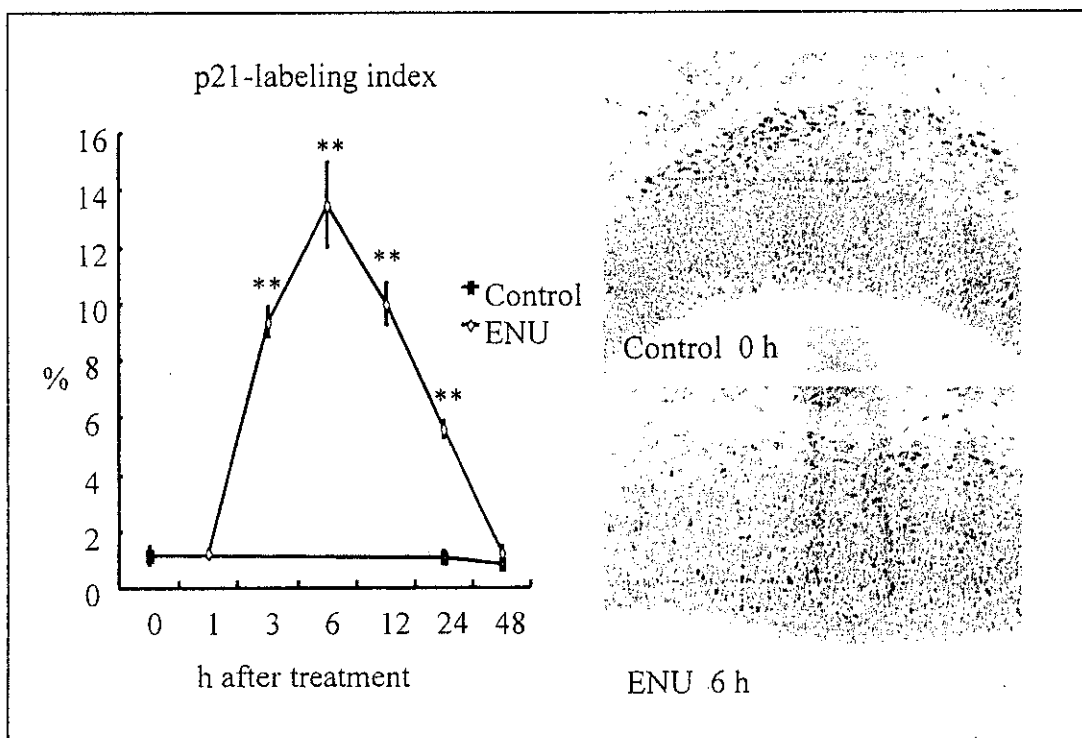
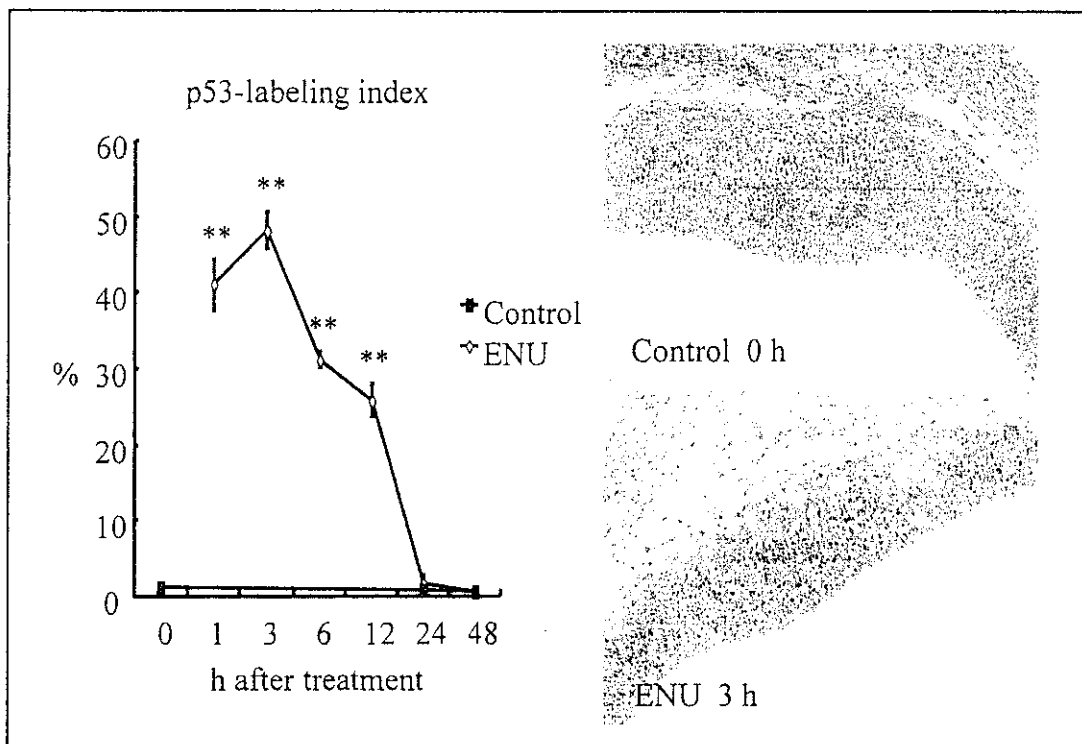


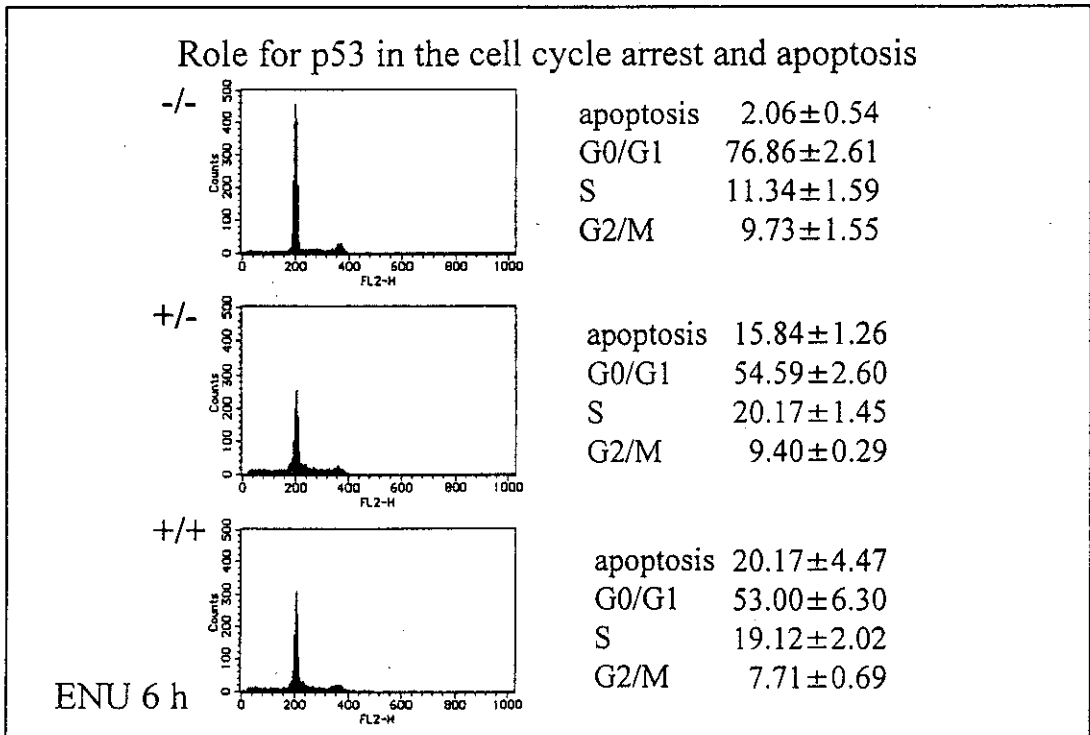
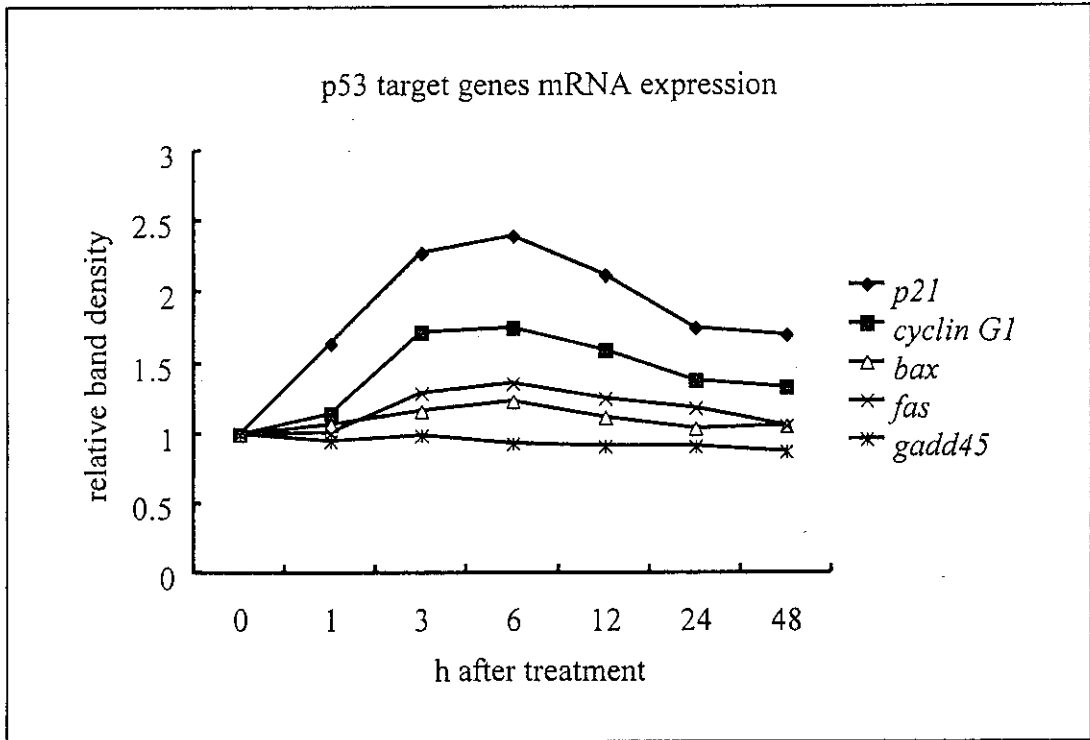
### Cell Cycle Distribution after ENU Exposure



### BrdU-incorporation assay



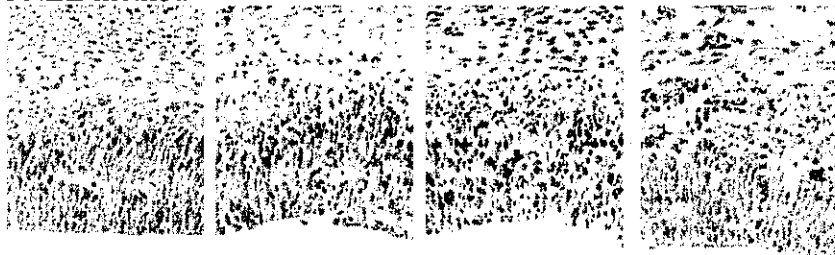




Mechanisms of 5-azacytidine (5AzC)-induced toxicity  
and regeneration  
in the central nervous system of rat fetuses

Apoptotic cells

○ TUNEL method



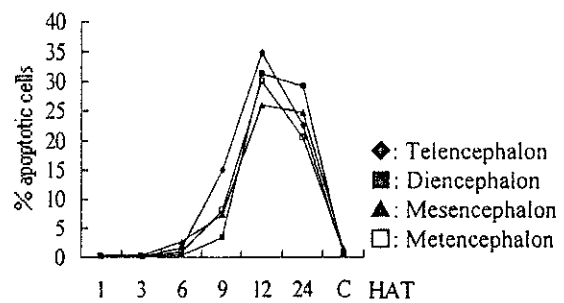
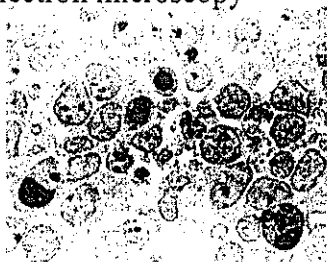
cont.

9

12

24

○ Electron microscopy



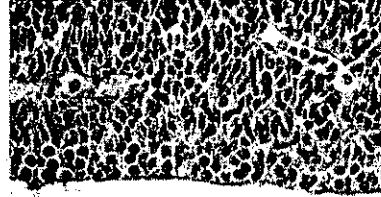


## Accumulation of mitotic cells

○ HE stain

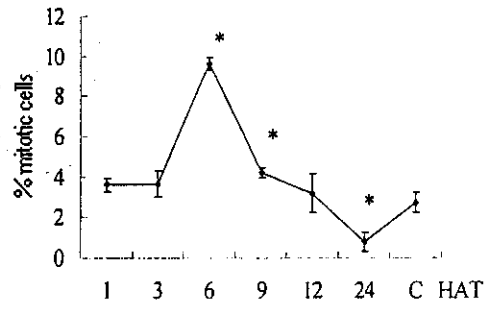


cont.



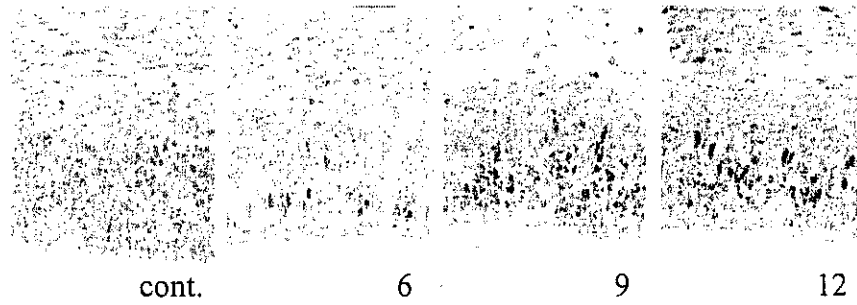
6 HAT

○ Electron microscopy

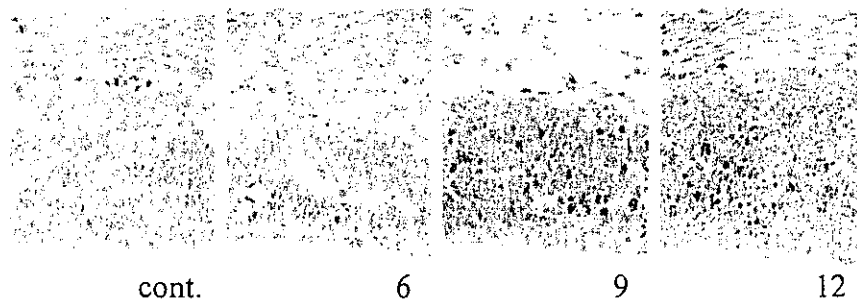


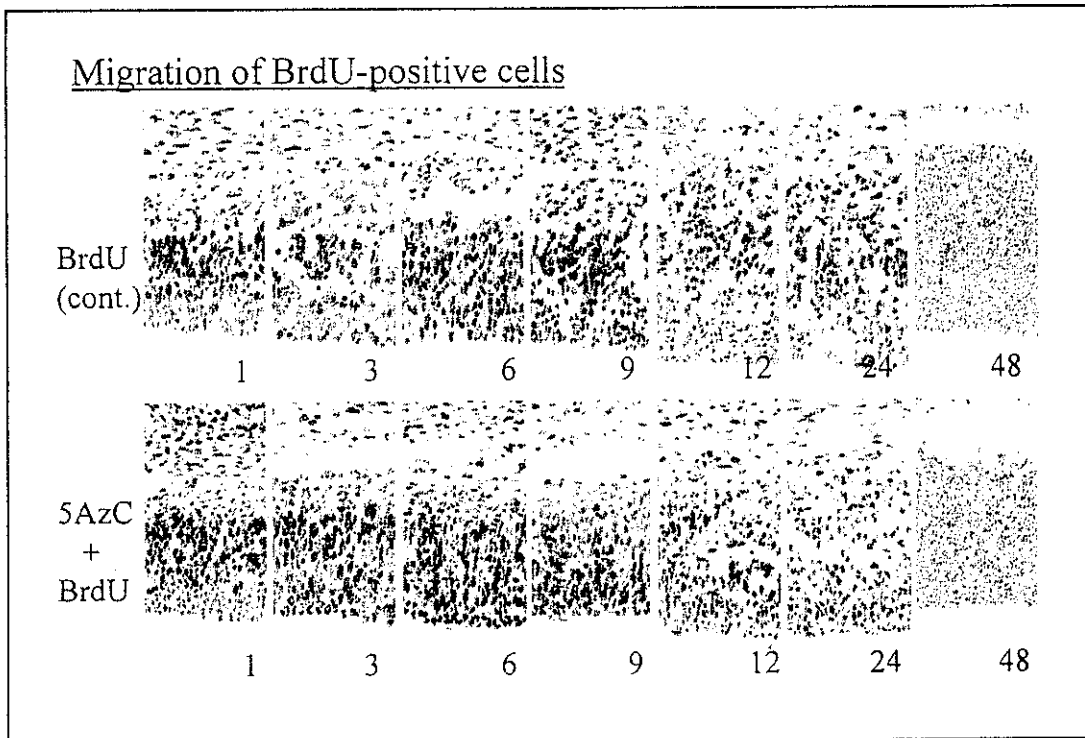
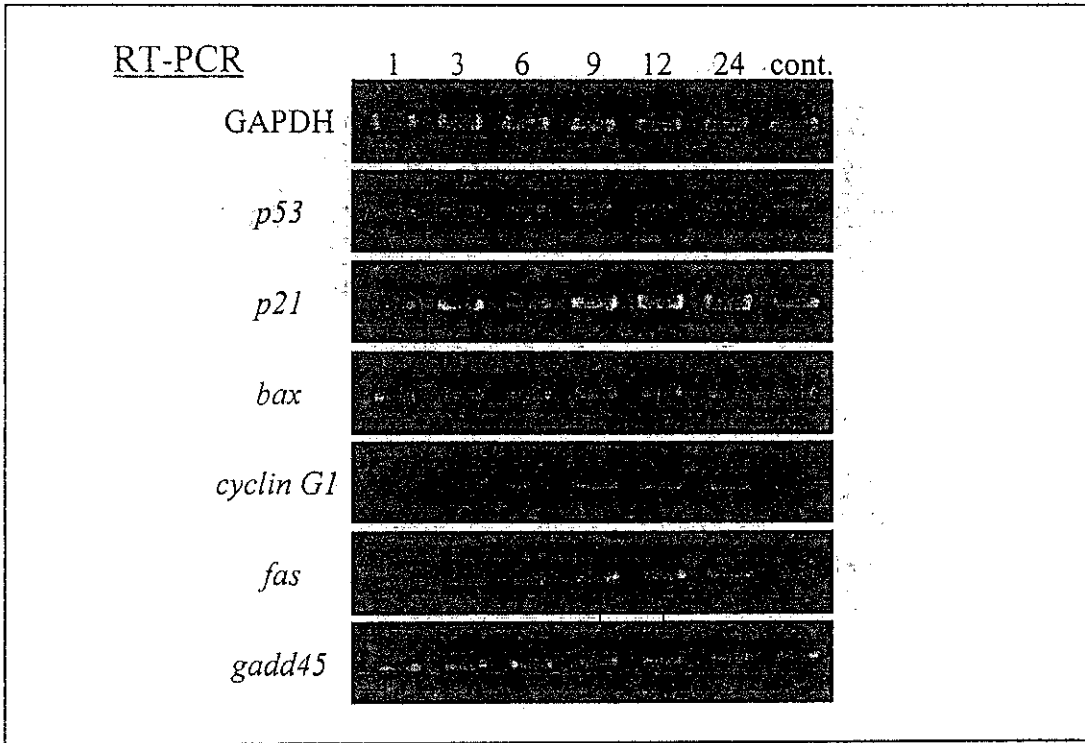
## Immunohistochemistry

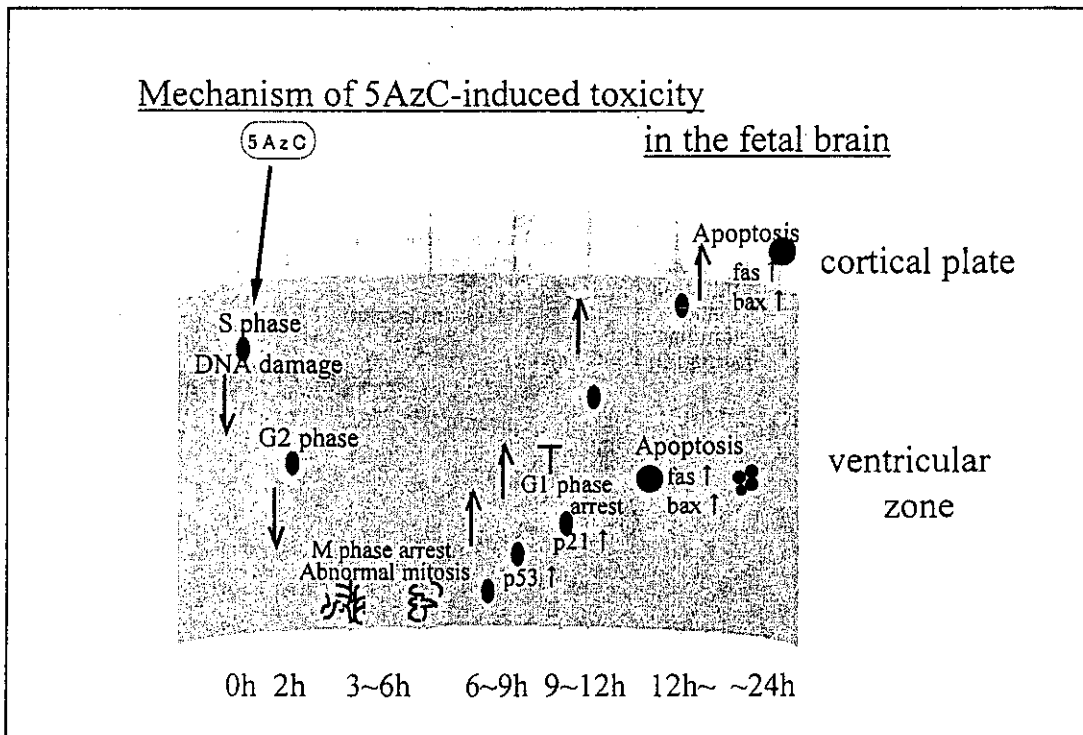
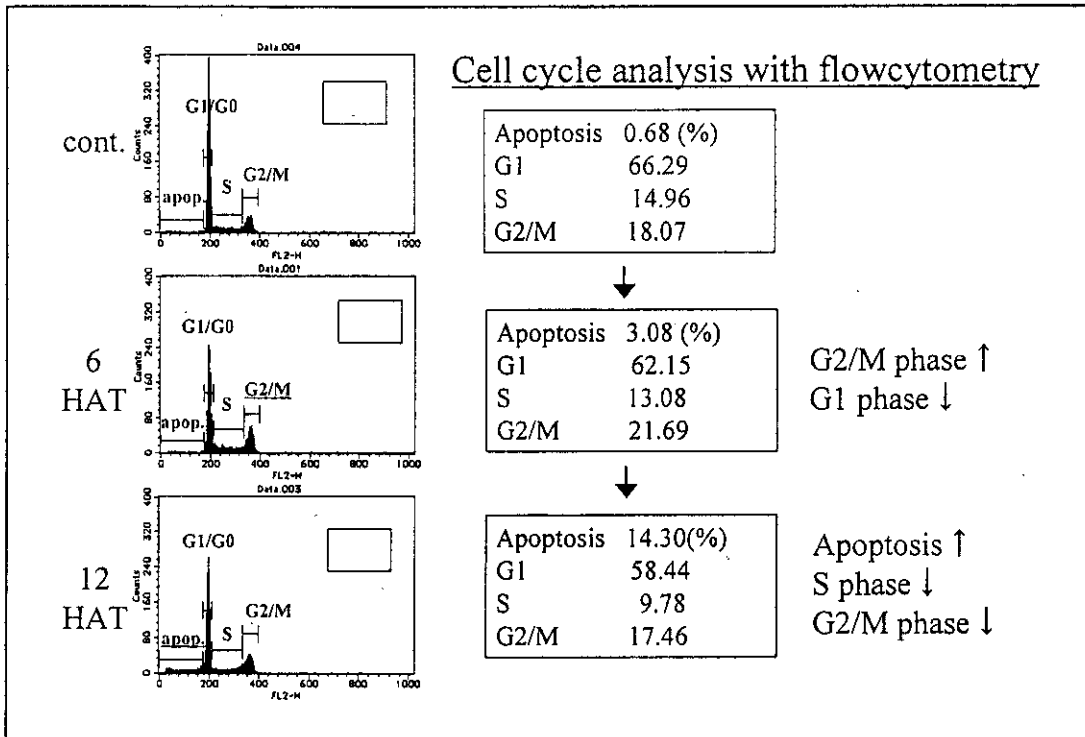
p53



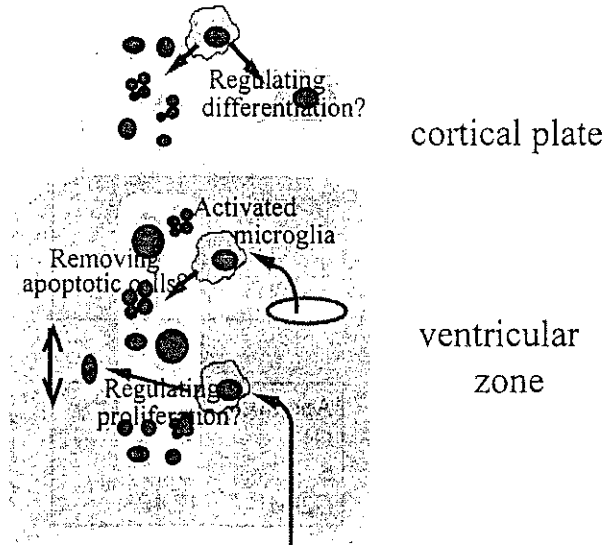
p21







Regeneration of injured fetal brain



Abnormal development of injured fetal brain

