

# PC12 세포와 A123.7 세포에서 차별적으로 발현되는 유전자의 검색\*

백승연\*\* · 양병환\*\*\* · 채영규\*\*†

## Screening of Differentially Expressed Genes between PC12 Cells and A123.7 Cells\*

Seung-Youn Baik, M.Sc.,\*\* Byung-Hwan Yang, M.D.,\*\*\* Young-Gyu Chai, Ph.D.\*\*†

### ABSTRACT

The cAMP-dependent protein kinase(PKA) is an intracellular enzyme with serine-threonine kinase activity that plays a key role in cell growth, differentiation, and apoptosis in eukaryotes. In order to understand the PKA signal transduction pathway regulating cell life cycle and identify its role, we focused on the characterization of up-/down-regulated genes by PKA using the differential display polymerase chain reaction. Seven differentially expressed sequence tags(DEST) have been obtained. Among these DESTs, 2 DESTs were homologous to the sequence of genes from BLAST search result. KC1 - 5 DEST that was up-regulated in A123.7 cells was highly corresponded to mouse apoptosis-related gene(MA-3) or mouse mRNA for topoisomerase inhibitor suppressed(TIS). MA - 3 was induced in various types of apoptosis, specially in NGF-deprived apoptotic PC12 cells. TIS was down-regulated in the RVC lymphoma cells incubated with topoisomerase inhibitor that induces DNA strand breakages. PG1 - 1 DEST that was highly expressed in PC12 cells was corresponded to *transposon Tn10* 3'-end. Transposon Tn10 was up-regulated in differentiated myeloblastic ML - 1 cells by 12 - O - tetradecanoylphorbol - 13 - acetate. This study illuminates that MA - 3/TIS was down-regulated by PKA activity, and *transposon Tn10* was up-regulated by it.

**KEY WORDS** : cAMP-dependent protein kinase(PKA) · A123.7 cell · PC12 cell · DD-PCR.

(regulatory subunit ; R<sub>2</sub>)  
(catalytic subunit ; C<sub>2</sub>)

가 , cAMP가  
가 , holoenzyme(R<sub>2</sub>C<sub>2</sub>)  
가  
가  
가 , PKA I PKA II  
가 , (Bramson 1983).  
I  
II  
(Cho - Chung 1995).  
PKA II가  
PKA I가  
(Ciardiello Tortora 1998).  
(epidermal growth factor ; EGF)  
mitogen - activated protein kinase(MAPK)  
(Kannan 1997).  
EGF cAMP PKA

서 론

Serine - threonine kinase 가 cAMP - dependent protein kinase(PKA) , PKA I PKA II (Taylor 1990 ; Montminy 1997). PKA 가 , (Bramson 1983). 1997 (Cho - Chung 1995). PKA II가 PKA I가 (Ciardiello Tortora 1998). (epidermal growth factor ; EGF) mitogen - activated protein kinase(MAPK) (Kannan 1997). EGF cAMP PKA

This study was supported by grant from the Industrial Technology Development Supply Plan of Ansan City.

Department of Biochemistry and Molecular Biology, College of Science, Hanyang University, Ansan, Korea

Department of Neuropsychiatry, College of Medicine, Hanyang University, Seoul, Korea

† : , 425 - 791 1271 (0345) 400 - 5513, (0345) 406 - 6316

MAPK . PKA I EGF  
 MAPK , PKA  
 II PKA I, ras raf - 1 MAPK  
 (Ciardiello Tortora 1998).  
 pheochromocytoma PC12  
 (nerve growth factor ; NGF)  
 (acetylcholine) (catecholamine)  
 (neurite)  
 (Greene Tischler 1976 ; Sc-  
 hubert Klier 1977), EGF  
 (Salomon 1995). , A123.7 PC12  
 pHL - REVB 가 PKA I  
 , PKA II 80%  
 (Scheibe 1991). A123.7 cAMP 가  
 , cAMP NGF  
 (Ginty 1991).  
 PKA tyrosine hydroxylase  
 (Nankova 1996), (neur -  
 onal nicotinic cholinergic receptor ; nAcChRs)  
 (Madhok 1995), PKA II  
 (choline acetyltransferase ; ChAT)  
 (vesicular acetylcholine transporter ; VAcHT)  
 (Wu 1984 ; Inoue 1995 ; Shimojo  
 1998).  
 reverse transcription - polymerase chain re -  
 action Liang Pardee differential  
 display polymerase chain reaction(DD - PCR) (1992)  
 , Alzheimer 가 ch -  
 olinergic gene locus  
 PC12 A123.7

## 실험 방법

### 1. 세포 배양

PC12 American Type Culture Co -  
 llection , PKA가 PC12 (A123.7)  
 Harvard Wagner .  
 PC12 A123.7 F12 - DMEM(Gibco/BRL)  
 10% fetal bovine serum(FBS)(Gibco/BRL) 5% ho -  
 rse serum 가 37 , 10% CO<sub>2</sub>  
 (Scheibe 1991).

### 2. RNA의 분리 및 정제

RNA 0.1% diethyl - pyrocarbonate(DE -  
 PC) . RNA Cho -  
 mczynski Sacchi (1987 ) RNAzol™B (Bi -  
 otecx) PBS 3  
 , 2ml RNAzol™B  
 , 15 , 0.1  
 . , 15  
 2 100% 가 , - 70  
 30 4 14,000rpm 20  
 . 70% RNA  
 DEPC - RNA UV/visible  
 260nm 280nm  
 O.D<sub>260nm</sub>/O.D<sub>280nm</sub> 1.9 RNase free - DNase I  
 / RNA  
 - 70

### 3. RNase free-DNase I 처리

RNase가 1.5ml RNA 50 µg, 0.25 µl  
 40U/µl RNase inhibitor(Promega), 10 µl RNase free -  
 DNase I(Promega), 5 µl 0.1 M Tris - HCl(pH 8.3), 5 µl  
 0.5 M KCl 5 µl 15mM MgCl<sub>2</sub> 50 µl  
 가 37 30  
 (pH 4.3)/ (3 : 1) 50 µl  
 14,000 rpm, 4 2  
 RNA

### 4. Formaldehyde 겔 전기영동

Formaldehyde 가 0.6g 5ml 10x  
 MOPS, 8.9ml formaldehyde, 0.5 µl ethidium bromide  
 가 , 5 µl RNA 15 µl RNA -  
 loading dye [ 0.72ml formamide, 0.16ml 10X MOPS, 0.26ml  
 37% formaldehyde, 0.18ml , 0.1ml 80% glycerol,  
 0.08ml bromophenol blue가 ]

### 5. Reverse transcription

cDNA 0.2 µg RNA, 20 µM dNTP, 20 pmole oligo  
 (d)T , [50mM Tris - HCl(pH 8.3), 75mM  
 KCl, 3mM MgCl<sub>2</sub>, 10mM DTT] 65 5  
 , 42 10 , 1 µl MMLV -  
 (reverse transcriptase ; 200 U/µl) 가 42  
 50 , 75 5 가 ,  
 oligo(d)T H - T<sub>12</sub>MA, H - T<sub>12</sub>MG H - T<sub>12</sub>MC( 1) ,

**Table 1.** Sequences of the primer for DD-PCR

Primer name	Primer sequence
H-AP1	5'-AAGCTTGATTGCC-3'
H-AP2	5'-AAGCTTCGACTGT-3'
H-AP3	5'-AAGCTTTGGTCAG-3'
H-T <sub>12</sub> MA	5'-AAGCTTTTTTTTTTMA-3'
H-T <sub>12</sub> MG	5'-AAGCTTTTTTTTTTMG-3'
H-T <sub>12</sub> MC	5'-AAGCTTTTTTTTTTMC-3'

3 cDNA 6 cDNA

### 6. Differential display-polymerase chain reaction (DD-PCR)

DD-PCR RNA Image Kit(GenHunter, Brookline, MA) (PC12/A123.7)

RNA 2 µl cDNA, 2 µM dNTP, 4pmole (H-AP)(GenHunter), 20pmole oligo(d)T (H-T<sub>12</sub>MA, H-T<sub>12</sub>MG, H-T<sub>12</sub>MC), 1 µl [<sup>35</sup>S] dATP (1,200 Ci/mmmole, Amersham), 1 unit Taq DNA (Perkin Elmer), [50mM KCl, 10mM Tris-HCl (pH 9.0 at 25 °C), 0.1% Triton X-100, 1.5mM MgCl<sub>2</sub>]

94 5 PCR 94 30  
, 40 2 annealing, 72 30  
40 , 72 10

H-AP1, H-AP2, H-AP3 가 ,  
oligo(d)T H-T<sub>12</sub>MA, H-T<sub>12</sub>MG

H-T<sub>12</sub>MC 9 가 가  
18 DD-PCR . DD-PCR PCR PCR 3

### 7. Polyacrylamide 겔 전기영동(PAGE)

Polyacrylamide(6%) Sigma coat coating  
. DD-PCR 3.5 µl 2 µl loading  
dye [0.25% bromophenol blue, 0.25% xylene cyanole FF, 15% Ficoll(Type 400 ; Pharmacia) ]

### 8. DEST의 획득과 재증폭

microcentrifuge 100 µl 15  
2 . 10  
µl 3 M sodium acetate, 5 µl glycogen(10mg/ml) 450 µl  
100% -70 30 , 4  
10 .

85% 10 µl PCR  
4 µl DNA, 20 µM dNTP,  
4 pmole H-AP , 20 pmole oligo(d)T , 1 unit Taq  
DNA , [50mM KCl, 10mM Tris-HCl  
(pH 9.0 at 25 °C), 0.1% Triton X-100, 1.5mM MgCl<sub>2</sub>]

94 5 , 94 30 , 40  
2 annealing, 72 30 40  
, 72 10 .

DD-PCR

. PCR 10 µl 가 ,

가 X-ray

### 9. 증폭된 DEST의 클로닝과 transformants 검색

cDNA pZEro-2<sup>TM</sup>  
(Invitrogen) . H-AP oligo(d)T  
HindIII 가 , DEST  
pZEro-2<sup>TM</sup> HindIII(BMS) , T4 DNA  
ligase(BMS) 16 3 . Liga-  
tion FSB XL1-Blue(*Escherichia coli*)  
competent cell 42 , 90 가 (tr-  
ansformation) . 1mM IPTG ka-  
namycin(25 µg/ml) 가 LB plate  
PCR .

### 10. DNA 염기서열 결정 및 분석

DNA Sanger Coulson chain termin-  
ation (1975) Amersham USB seque-  
ncing 2.0 kit(Sequenase 2.0) , - [<sup>35</sup>S] dATP  
(1,200 Ci/mmmole, Amersham) DNA labeling .  
DEST DNA 10 µg , M13 re-  
verse forward 65 2 annealing ,  
35 - [<sup>35</sup>S] dATP, DTT(0.1 M), labe-  
ling , Sequenase 2.0 가 2~5 la-  
beling , termination 가 lab-  
eling 37 5 , 4 µl  
가 . sequencing  
Phosphoimage analyzer(Bio-Rad)  
BLAST  
GenBank (Alts-  
chu 1997 ; http : //www.ncbi.nlm.nih.gov/BLAST).

### 11. Northern analysis

Formaldehyde(1.2%) RNA , RNA  
10X SSC 10 (3 ) ,

upward capillary blotting 12 nitrocellulose  
 filter RNA가 filter UV - crosslinker  
 (Bio - Rad) cross - linking Probe BLAST  
 95% KC1 - 5 DEST  
 PCR ECL kit(Amercham) horsera -  
 dish peroxidase labeling , 6 hybridization  
 , 42 [ 6 M urea, 0.4% SDS, 0.5x  
 SSC ) 20 2 [ 2X SSC ]  
 5 2 , ECL - detection solution(Amer -  
 sham) X - ray 1

6% denaturing 1  
 가 12 DEST  
 DEST DEST  
 DEST DEST  
 A123.7 P PC12 K  
 antisense oligo(d)TA, G C  
 1, 2 3 sense H - AP1, 2  
 3  
 DEST가 2 가 1 2  
 KC1 - 1, KC1 - 2, KC1 - 3, KC1 -  
 4, KC1 - 5, KC2 - 1, KC3 - 1, KC3 - 2, KA3 - 1, KA1 - 1, PG1 -  
 1 PG1 - 2 12 DEST ( 1).

## 결 과

### 1. DD-PCR과 DEST의 선택

PC12 A123.7 mRNA  
 sense H - AP1, H - AP2 H -  
 AP3 ( 1) , antisense H - T<sub>12</sub>MA,  
 H - T<sub>12</sub>MG H - T<sub>12</sub>MC ( 1) PCR ,

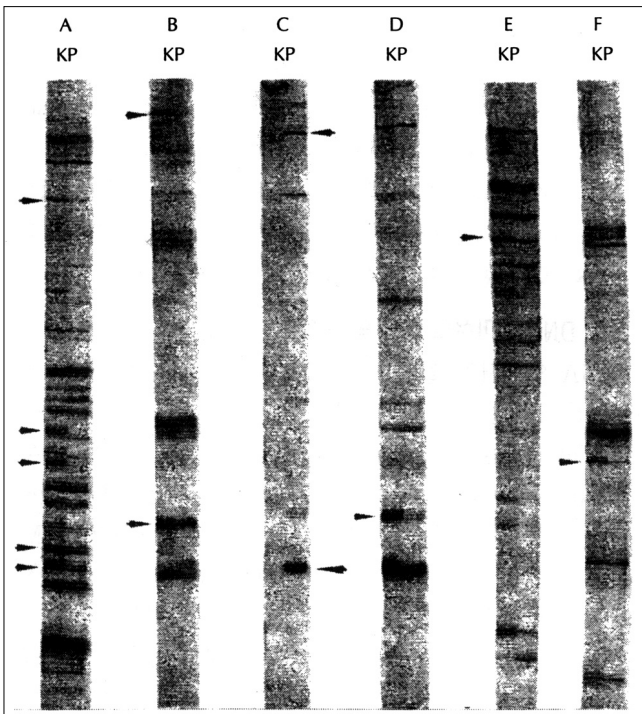


Fig. 1. Differentially expressed mRNAs between in PC12 cells and A123.7 cells. The total RNAs were purified from A123.7 cell and PC12 cell. DD-PCR reaction was done with  $\alpha$ -[<sup>35</sup>S] dATP and primers of H-T<sub>12</sub>C and H-AP1 in panel A, H-T<sub>12</sub>C and H-AP3 in panel B, H-T<sub>12</sub>G and H-AP1 in panel C, H-T<sub>12</sub>A and H-AP1 in panel D, H-T<sub>12</sub>C and H-AP2 in panel E, and H-T<sub>12</sub>A and H-AP3 in panel F. The radio-labelled DD-PCR products were electrophoresised on 6% polyacrylamide gel. K stands for A123.7 cell and P for PC12 cell. Arrow heads indicate differentially expressed mRNAs.

### 2. DEST의 획득과 재증폭

DD - PCR 12 DEST  
 DNA ,  
 ( 2). 11 DEST  
 , 6% denaturing  
 1.5% 가

### 3. DEST의 클로닝과 염기서열 결정 및 분석

DEST pZerO - 2 (Invitr -  
 ogen)  
 HindIII DEST ,  
 pZerO - 2 HindIII ligation 7 DEST

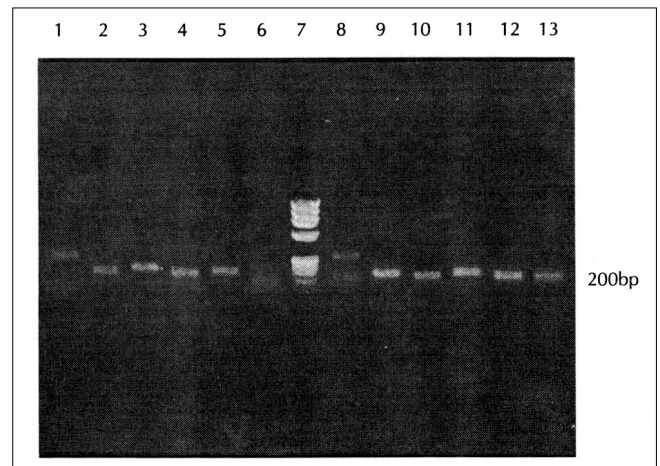


Fig. 2. Reamplification of differentially expressed cDNA fragments. Purified cDNA fragments out of polyacrylamide gel were reamplified by PCR using same primer sets that used in DD-PCR. Lane 1 ; KC2-1, lane 2 ; PG1-1, lane 3 ; KC1-2, lane 4 ; PG1-2, lane 5 ; KC1-3, lane 6 ; KC3-1, lane 7 ; molecular weight standard ( 174 DNA was digested with HaeIII), lane 8 ; KC1-1, lane 9 ; KA1-1, lane 10 ; KC1-5, lane 11 ; KA3-1, lane 12 ; KC1-4, and lane 13 ; KC3-2.

**Table 2.** Result of DESTs and BLAST search

DEST	Size (bp)	BLAST result
KC1-5	142	Mouse mRNA for topoisomerase-inhibitor suppressed (TIS)
KC1-5	142	Mouse apoptosis-related gene (MA-3)
KC1-3	169	Unknown gene
KC1-1	165	Unknown gene
KA3-1	158	Unknown gene
KC1-2	157	Unknown gene
KC2-1	148	Unknown gene
PG1-1	190	Transposon Tn10 DNA

**dbj:D86344:D86344 Mouse mRNA for Topoisomerase-inhibitor suppressed, complete CDs, Length=2196**

Score=190 bits (96), Expect=4e-47  
 Identities=115 / 120 (95%), Positives=115 / 120 (95%), Gaps=1 / 120 (0%)

Query:5 ttgattgcccttaaatattagacagtaagtaaatagtggtgctggaacatctattttct 64  
 Sbjct:2071 ttgattggtcctaaatattagacagtaagtaaat-ggtggtggaacatctattttct 2129

Query:65 acaaaactggaaaaacgaaccgggttctacaaaaatgtacaacaaaataaacatgtgaa 124  
 Sbjct:2130 acaaaactggaaaaacgaaccgggttctacaagaatgtacagcaaaaataaacatgtgaa 2189

**dbj:D50465:MUSMA3CDNA Mouse MA-3 (apoptosis-related gene) mRNA, Complete cds, Length=2181**

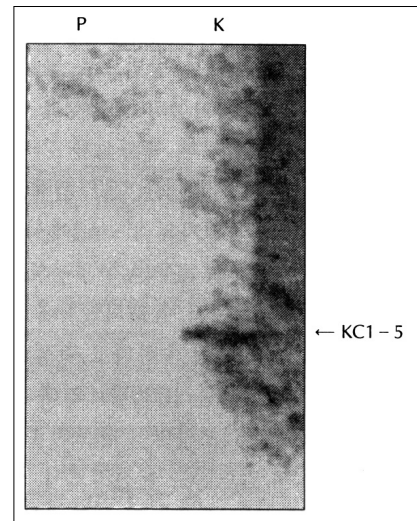
Score=182 bits (92), Expect=1e-44  
 Identities=114 / 120 (95%), Positives=114 / 120 (95%), Gaps=1 / 120 (0%)

Query:5 ttgattgcccttaaatattagacagtaagtaaatagtggtgctggaacatctattttct 64  
 Sbjct:2055 ttgattggtcctaaatattagacagtaagtaaat-ggtggtggaacatctattttct 2113

Query:65 acaaaactggaaaaacgaaccgggttctacaaaaatgtacaacaaaataaacatgtgaa 124  
 Sbjct:2114 acaaaactggaaaaacgaaccgggttctacaagaatgtacagcaaaaataaacatgtgaa 2173

**Fig. 3.** BLAST search result of KC1-5 DEST. KC1-5 DEST was differentially expressed in A123.7 cells. KC1-5 DEST was identical two genes, mouse apoptosis-related gene, MA-3 and mouse mRNA for topoisomerase inhibitor suppressed, TIS.

KC1 - 1, KC1 - 2, KC1 - 3, KC1 - 5, KC2 - 1, KA3 - 1  
 PG1 - 1 , , BLAST  
 (Altschul 1997 ; 2). KC1 - 5  
 DEST topoisomerase  
 , TIS (Onishi Kizaki 1996)  
 , MA - 3 (Shibahara 1995) 95% 가  
 , DNA 98% ,  
 99.6% 가 ( 3).  
 PG1 - 1 DEST *transposon Tn10* DNA, 3' - end 98%  
 가 ( ).  
 5 DEST BLAST  
 가 ( 2).



**Fig. 4.** Northern analysis of KC1-5 DEST. RNA were isolated from PC12 cells and A123.7 cells. KC1-5 DEST was differentially expressed in A123.7 cells. P stands for PC12 cells, K for A123.7 cells.

4. Northern analysis를 통한 DEST의 확인

A123.7 PC12  
 7 DEST , ,  
 PKA MA - 3 TIS KC1 - 5  
 DEST Northern analysis PC12  
 , A123.7 ( 4).  
 MA - 3/TIS KC1 - 5 DEST PKA

고 찰

PC12 A123.7  
 DD - PCR 7  
 DEST . DEST 6 A123.7  
 , 1 DEST PC12  
 . KC1 - 5 PG1 - 1 DEST BLAST  
 90% 가 , 5  
 DEST BLAST  
 가 .  
 KC1 - 5 DEST BLAST 2 95%  
 , MA - 3 (Shibahara 1995), to -  
 poiserase , RVC lymphoma  
 , TIS (Onishi Kizaki 1996).  
 DNA  
 98% 가 ,  
 99.6% 가 . 2 KC1 -

5 DEST BLAST MA - 3 DNA  
*TIS* 3' - end 95% (human genome)  
 DD - PCR cDNA가 3' - end , PC12 transposonal factor (Wang  
 가 KC1 - 5 DEST MA - 3/ 1998). Tn10 - like myeloblastic leukemia  
*TIS* familly ML - 1 12 - O - tetradecanoylphorbol - 13 - ac -  
 MA - 3 Shibahara (1995) DD - PCR etate(TPA) ML - 1  
 , PC12, B cell, DD - PCR , TPA  
 T cell thymocyte , PC12 ML - 1 (Wang 1998). , Tn10  
 , NGF . Yao , PKA  
 (1998) , NGF 가 MA - 3/TIS  
 , MAP ERK , PKA PKA PKA , transposon Tn10  
 가 PKA , PC12 NGF PKA PKA  
 PKA , A123.7 PKA MA - 3/TIS transposon Tn10 PKA  
 MA - 3 . Shibahara (1995)  
 , MA - 3  
 , A123.7 PC12

## 결 론

MA - 3  
 , cAMP - dependent protein kinase(PKA)  
 , PKA  
 TIS DNA 가 topoisomerase PKA , PKA  
 가 RVC lymphoma , PKA 가  
 topoisomerase 가 . PKA PC12 , A123.7  
 (Onishi Kizaki 1996). Topoisomerase PC12 differ -  
 topoisomerization DNA , ential display polymerase chain reaction  
 , topoisomerase I camptothecin 7 differential expressed sequence tags(DEST)  
 가 DNA , topoisomerase II . 5 DEST BLAST  
 etopside 가 DNA 가 cleavable co - , 2 DEST 90%  
 mplex (Hsiang 1985). *TIS* 가 . A123.7  
 cleavable complex topoisomerase II 가 KC1 - 5 DEST  
 ICRF - 154 . Ga - MA - 3 topoisomerase  
 rcia - Bermejo (1998) , PKA topoiso - TIS 95% . MA - 3  
 merase II , NGF  
 PKA가 A123.7 , topoisomerase II가 PC12 . TIS  
*TIS* 가 . MA - 3 DNA 가 topoisomerase 가  
 , (Shibahara topoisomerase , to -  
 1995). *TIS* , KC1 - 5 DEST Northern analysis A123.7  
 , (On - . PC12 PG1 -  
 ishi 1998). 1 DEST transposon Tn10 98% . Tr -  
 PG1 - 1 DEST PC12 , transposons, ansposon Tn10 12 - O - tetradecanoylhorbol - 13 - acetate  
 Tn10 DNA 98% (Wang 1998). Tn10 myeloblastic ML - 1  
 DNA , MA - 3/TIS PKA

, transposon Tn10 PKA

중심 단어 : cAMP - (PKA) · PC12  
· A123.7 · DD - PCR.

감사의 글

---

## 참고문헌

---

- Altschul SF, Madden TL, Schfer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) : Gapped BLAST and PSI-BLAST : A new generation of protein database search programs. *Nucleic Acids Res* 25 : 3389-3402
- Bramson HN, Kaiser ET, Mildvan AS (1983) : Mechanistic studies of cAMP-dependent protein kinase actions. *Crit Rev Biochem* 15 : 93-124
- Cho-Chung YS, Pepe S, Clair T, Budillon A, Nesterova M (1995) : cAMP-dependent protein kinase : Role in normal and malignant growth. *Crit Rev Oncol Hematol* 21 : 33-61
- Chomczynski P, Sacchi N (1987) : Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162 : 156-159
- Ciardello F, Tortora G (1998) : Interactions between the epidermal growth factor receptor and type I protein kinase A : Biological significance and therapeutic implications. *Clin Cancer Res* 4 : 821-828
- Garcia-Bermejo L, Perez C, Vilaboa NE, de Blas E, Aller P (1998) : cAMP increasing agents attenuate the generation of apoptosis by etoposide in promonocytic leukemia cells. *J Cell Sci* 111 : 637-644
- Ginty DD, Glowacka D, DeFranco C, Wagner JA (1991) : Nerve growth factor-induced neuronal differentiation after dominant repression of both type I and type II cAMP-dependent protein kinase activities. *J Biol Chem* 266 : 15325-15333
- Greene LA, Tischler AS (1976) : Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc Natl Acad Sci USA* 73 : 2424-2428
- Hsiang YH, Hertzberg R, Hecht S, Liu LP (1985) : Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 260 : 14873-14878
- Inoue H, Li YP, Wagner JA, Hersh LB (1995) : Expression of the choline acetyltransferase gene depends on protein kinase A activity. *J Neurochem* 64 : 985-990
- Kannan S, De Santis M, Lohmeyer M, Riese DJ, Smith GH, Hynes NE, Seno M, Brandt R, Bianco C, Persico MG, Kenney N, Normanno N, Martinez-Lacaci I, Ciardello F, Stern DF, Gullick WJ, Salomon DS (1997) : Cripto enhances the tyrosine phosphorylation of Shc and activates mitogen-activated protein kinase (MAPK) in mammary epithelial cells. *J Biol Chem* 272 : 3330-3335
- Liang P, Pardee AB (1992) : Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* 257 : 967-71
- Madhok TC, Matta SF, Sharp BM (1995) : Nicotine regulates nicotinic cholinergic receptors and subunit mRNAs in PC12 cells through protein kinase A. *Brain Res Mol Brain Res* 32 : 143-150
- Montminy M (1997) : Transcriptional regulation by cyclic AMP. *Annu Rev Biochem* 66 : 807-822
- Nankova B, Hiremagalur B, Menezes A, Zeman R, Sabban E (1996) : Promoter elements and second messenger pathways involved in transcriptional activation of tyrosine hydroxylase by ionomycin. *Brain Res Mol Brain Res* 35 : 164-172
- Onishi Y, Hashimoto S, Kizaki H (1998) : Cloning of the TIS gene suppressed by topoisomerase inhibitors. *Gene* 215 : 453-459
- Onishi Y, Kizaki H (1996) : Molecular cloning of the genes suppressed in RVC lymphoma cells by topoisomerase inhibitors. *Biochem Biophys Res Commun* 228 : 7-13
- Salomon DS, Brandt R, Ciardello F, Normanno N (1995) : Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 19 : 183-232
- Sanger F, Coulson AR (1975) : A rapid method for determining sequences in DNA by primed synthesis with polymerase. *J Mol Biol* 94 : 441-448
- Scheibe RJ, Moeller-Runge I, Mueller WH (1991) : Retinoic acid induces the expression of alkaline phosphatase in P19 teratocarcinoma cells. *J Biol Chem* 266 : 21300-21305
- Schubert D, Klier FG (1977) : Storage and release of acetylcholine by a clonal cell line. *Proc Natl Acad Sci USA* 74 : 5184-5188
- Shibahara K, Asano M, Ishida Y, Aoki T, Koike T, Honjo T (1995) : Isolation of a novel mouse gene MA-3 that is induced upon programmed cell death. *Gene* 166 : 297-301
- Shimojo M, Wu D, Hersh LB (1998) : The cholinergic gene locus is coordinately regulated by protein kinase A II in PC12 cells. *J Neurochem* 71 : 1118-1126
- Taylor SS, Buechler JA, Yonemoto W (1990) : cAMP-dependent protein kinase : Framework for a diverse family of regulatory enzymes. *Annu Rev Biochem* 59 : 971-1005
- Wang Y, Gong B, Dai W, Lu L (1998) : Identification of immediate early genes during TPA-induced human myeloblastic leukemia ML-1 cell differentiation. *Gene* 216 : 293-302
- Wu N, Armstrong I, Wagner J (1984) : Genetic evidence that chloroadenosine increases the specific activity of choline acetyltransferase in PC12 via modulation of an adenosine-dependent adenylyl cyclase. *Neuroscience* 13 : 1365-1371
- Yao H, York RD, Misra-Press A, Carr DW, Stork PJS (1998) : The cyclic adenosine monophosphate-dependent protein kinase (PKA) is required for the sustained activation of mitogen-activated kinase and gene expression by nerve growth factor. *J Biol Chem* 273 : 8240-8247