

CD99 가

The increased expression of CD99 in a differentiated neuroblastoma cell line

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= Abstract =

Background: The human *mic2* gene is a pseudoautosomal gene that encodes a cell surface antigen, CD99. High levels of CD99 constitute a tumor marker in Ewing's sarcoma (ES). We have recently demonstrated that CD99-induced apoptosis occurs only in undifferentiated ES cells, not in differentiated ES cells, raising the possibility of the involvement of CD99 in neural ontogeny. **Methods:** To elucidate the relations between the expression of CD99 and the differentiation of neural cells and the mechanism by which the expression of CD99 is regulated, we analyzed the differential patterns of CD99 expression in SH-SY5Y by treatment of 12-O-tetradecanoyl-13-phorbol acetate (TPA) and retinoic acid. In addition, to explore the transcriptional activity of CD99 during neural cell differentiation, SH-SY5Y cells were transiently transfected with a CD99 promoter-driven luciferase construct, and treated with the inducers. **Results:** In immunoblotting and flow cytometry, the expression level of CD99 was increased on differentiated SH-SY5Y cells induced by TPA and retinoic acid. The luciferase activity was elevated by the treatment with TPA, known to mature SH-SY5Y cells toward a sympathetic neuronal lineage, whereas retinoic acid inducing a sympathetic chromaffin lineage displayed little effect. **Conclusion:** The result indicates that CD99 might be expressed only on cells maturing toward a neuronal lineage among differentiating primitive neuronal cells. In addition, the expression of CD99 seems to be regulated at the transcriptional level during the differentiation.

Key Words: Human, CD99, Expression, Neuroblastoma, SH-SY5Y, Differentiation, Promotor

CD99 32-kDa

Sertoli
(4). CD99

(1-3). *Mic2* CD99

(Ewing's sarcoma),
(neuroectodermal tumor)

mic2 X Y
pseudoautosomal region

mic2 X

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(5). CD99, CD99, SH-SY5Y, neuropeptide Y, TPA
 가 maturation (6), (15,16). 가
 (7) (8) T (TCR) MHC TPA protein kinase C (PKC)
 (9). CD99 SH-SY5Y PKC가 (17), TPA
 가 ES
 (10). , CD99가 가
 (4). SH-N-SH (neuroblastoma) SY5Y TPA retinoic acid SH-
 CD99 CD99 promoter activity
 가 (N-type), (I-type)
 (S-type) SK-N-SH N- SH-SY5Y
 가 가 *in vitro*
 1. American Type Culture Collection (ATCC; Rockville, MD)
 , 10% 가
 가 phorbol ester, 12-O-tetradecanoyl-13-phorbol acetate (TPA) Dulbecco's modified Eagle's medium (DMEM)
 retinoic acid (11), Retinoic acid TPA ,
 SH-SY5Y retinoic acid (12-14). 10µM retinoic acid (all-trans: Sigma) 16nM TPA
 4 7 . MHC class I W6/32
 (13). enolase(NSE) ATCC
 가 GAP43 retinoic acid 가 (horseradish peroxidase)가 - IgG Zymed
 , neuro peptide Y , FITC (fluorescein isothiocyanate)가
 catecholamine - IgG Dako . CD99
 (13). retinoic acid DN 16 YG32 DiNonA Inc, Korea
 trkB (14) chromaffin
 - neurotrophin (BDNF) 2.
 RA trkB 가 SH-SY5Y PBS IMØ
 BDFB chromaffin (50 mM Tris, pH7.5, 0.5% NP-40, 5 mM EDTA, 150 mM NaCl, 1mM PMSF)
 , SH-SY5Y TPA 15,000 rpm 10 4
 가 . NSE, GAP43, PAGE , 5 , 10% SDS- 25 mV

18 software(Becton Dickinson)

5% (w/v) 가 TBST (Tris- buffered saline-Tween 20) 1

DN16 YG32

TBST 10 3

가 - IgG 45 1. SH-SY5Y CD99

TBST 10 3

ECP (Amersham) O-mat AR CD99 T

(Kodak) , (18)

3. (4,10).

retinoic acid TPA

(FACScan, 가 , ,

Becton-Dickinson, Mountain View, CA) 가

1×10^6 $50 \mu\text{l}$ 가 (16,19,20).

; , ; 1:1000) CD99

4 1

PBS 2 FITC SH-SY5Y CD99

IgGAM 4°C SH-SY5Y

30 2 retinoic acid TPA 4 7 , CD99

FITC가

FACScan Retinoic acid 4 CD99

(mean fluorescence intensity; MFI) 10^4 , 7 CD99 가

FACScan software(Becton Dickinson) TPA CD99

list mode retinoic acid 4

C30

Fig. 1. Western blotting analysis for human CD99 expression in SH-SY5Y cells after the treatment of differentiation inducing agents. SH-SY5Y cells were treated using TPA and retinoic acid for 4 days or 7days, and were used for western blotting to measure expression level of CD99. (T. thymocyte cell extract, C. DMSO control, RA. retinoic acid (10 uM), TPA. phorbol-12-myristate-13-acetate (16nM)).



Fig. 2. Flow cytometric analysis of up-regulation of CD99 and MHC class I molecule in SH-SY5Y by treatment of TPA. (secondary antibody only, CD99 expression in DN16 antibody treated SH-SY5Y, MHC class I expression in W6/32 antibody treated SH-SY5Y).

가 , 7 promotor , CD99
 가 (Fig. 1). Retinoic acid , CD99
 TPA SH-SY5Y 가
 (data not shown). 가 (21).
 retinoic acid TPA
 SH-SY5Y CD99 가 , CD99
 가 .
 CD99 CD99
 . CD99 , northern analysis ,
 가 . SH-SY5Y - 1654 +123 CD99 pro-
 CD99 가가 moter reporter luciferase가
 가 (p(-1654/+123)) , CD99
 SH-SY5Y 7 TPA CD99 CD99 promoter acitivity
 (FACS) (fig. 3). CD99 가
 . TPA 가 Jurkat , Western
 SH-SY5Y CD99 가 CD99 가 ,
 , CD99 가 CD99 가
 MHC class I Northern data . , CD99
 (Fig. 2). HeLa, IM9, BJAB CD99
 2. SH - SY5Y 가 , CD99 promoter
 CD99 promoter acitivity CD99 p(-1654/+123) transient
 transfection activity , CD99
 promoter activity가 가 Northern
 CD99 가 CD99 mRNA
 가 . SH-SY5Y
 CD99가 CD99 promoter
 . CD99 activity HeLa (data not

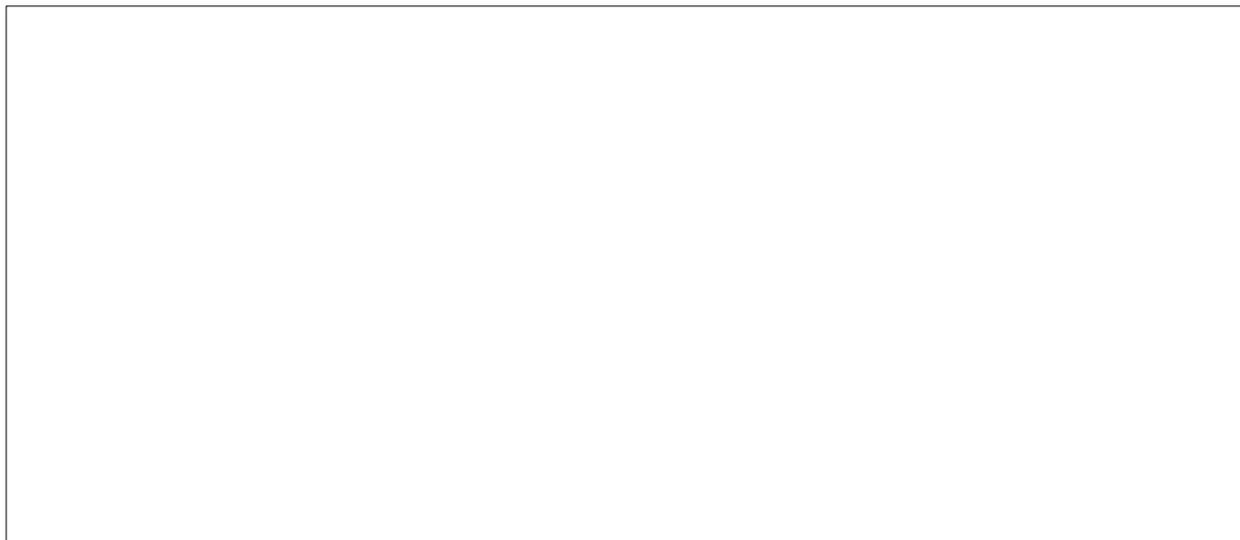


Fig. 3. Analysis of the CD99 expression in various cell lines. (A) Expression patterns of endogenous CD99 RNA. Total RNA was prepared from nonlymphoid (293, 293T, and HeLa) and lymphoid (BJAB, IM9, and Jurkat) cell lines, and 50 μ g of RNA from each cell line was separated by electrophoresis, and analyzed. The expression level of glyceraldehyde-3 phosphate dehydrogenase (GAPDH) of each cell line is shown in the lower panel as an internal control. (B) Expression pattern of endogenous CD99 protein. Cell extract was prepared, and 60 μ g of total protein was separated by SDS PAGE method. For the detection of CD99 molecules, the purified form of DN16 antibody (DiNonA Inc, Korea) was used. The expression level of Calnexin is shown as an internal control in the lower panel. (C) The relative activities of the reporter construct containing the CD99 promoter region between -1654 and +123, p(1654/+123)luc. The relative luciferase activity of CD99 promoter activity in each cell line is shown as fold induction relative to that of the promoterless luciferase construct, p(0)luc. To normalize transfection variation within the cell lines, the construct containing the TK promoter-driven Renilla luciferase (pRLTK) was cotransfected, and the activities of firefly and Renilla luciferases are measured sequentially from a single sample using dual-luciferase reporter assay system (Promega). The mean values + S.D. from at least more than three experiments are shown. The expression level of surface CD99 molecules was measured by FACS analysis, and is indicated as the relative antigen intensity [R.A.I.] in parenthesis at the bottom. The average of two independent experiments is shown.

shown).
 CD99
 CD99 activity
 CD99 TPA
 retinoic acid SH-SY5Y
 CD99 p(-1654/+123)
 , CD99
 retinoic acid TPA
 TPA SH-SY5Y 가
 CD99
 SH-SY5Y TPA TPA
 가 CD99 CD99
 가

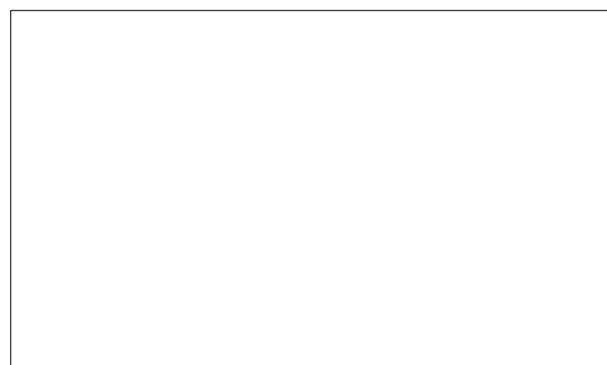


Fig. 4. Effect of retinoic acid and TPA on CD99 promoter activity in SH-SY5Y. SH-SY5Y were transiently transfected with p (-1654/+123). Substances were applied 1 day after transfection. Cells were collected for luciferase assay 48hrs post transfection. The solvent DMSO served as a control. Values represent means of three independent experiments. Bars are + SEM.

(Fig. 4). , TPA 4 CD99 . Chromaffin lineage retinoic acid , neuronal differentiation lineage TPA CD99 CD99가 CD99 가 , CD99 CD99가 SH-SY5Y CD99 TPA 가 (6-8). , CD99 MHC class I (22) , CD99 MHC class I 가 CD99 가 SH-SY5Y가 TPA 가 가 (10). CD99가 CD99 가가 CD99 promotor luciferase assay CD99 promotor luciferase construct SH-SY5Y TPA CD99 promotor 가 CD99 SH-SY5Y (neuroblastoma) SH-N-SH CD99 SH-SY5Y 가 TPA 가 (23) TPA sympathetic activity 가 CD99 neuronal differentiation lineage , retinoic acid sympathetic chromaffin lineage SH-SY5Y TPA retinoic acid CD99 . 1 SH-SY5Y 3 , 1 (neutrite) (data not shown). retinoic acid 가 CD99

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