

CD99

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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

mic2 X Y

pseudoautosomal region

mic2 X

(1-3). *Mic2*

Sertoli

(4). CD99

CD99

(Ewing's sarcoma),

(neuroectodermal tumor)

110-799, 28

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(5). CD99, CD99 maturation (6), (7) (8) (TCR) MHC (9). CD99 SH-SY5Y (10). , ES CD99가 , SH-SY5Y TPA (15,16). 가 protein kinase C (PKC) (17), TPA PKC가 .

(4). SH-N-SH (neuroblastoma) 가 (N-type), (S-type) (I-type) . SK-N-SH N- SH-SY5Y 가 가 . *in vitro* 1. American Type Culture Collection (ATCC; Rockville, MD) , 10% 가 Dulbecco's modified Eagle's medium (DMEM) . Retinoic acid TPA , 10 μ M retinoic acid (all-trans: Sigma) 16nM TPA 4 7 . MHC class I W6/32 ATCC (horseradish peroxidase)가 - IgG Zymed , FITC (fluorescein isothiocyanate)가 - IgG Dako . CD99 DN 16 YG32 DiNonA Inc, Korea .

(13). enolase(NSE) 가 GAP43 retinoic acid 가 , neuropeptide Y catecholamine (13). retinoic acid trkB (14) chromaffin - neurotrophin (BDNF) RA trkB 가 BDNF chromaffin . , SH-SY5Y TPA 15,000 rpm 10 4 가 5 , 10% SDS- 25 mV

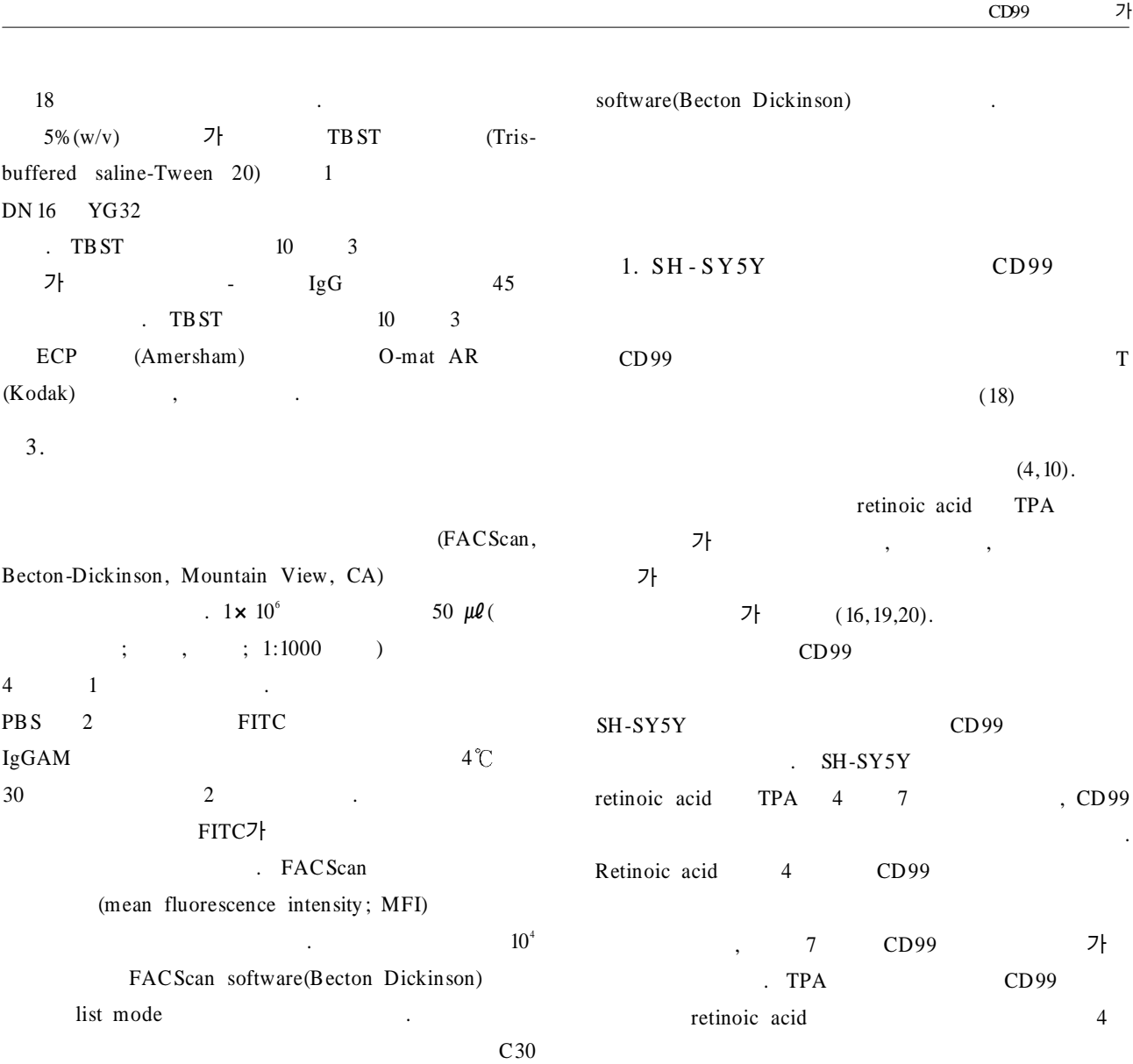


Fig. 1. Western blotting analysis for human CD99 expression in SH-SY5Y cells after the treatment of differentiation inducing agents. SH-SY5Y cells were treated using TPA and retinoic acid for 4 days or 7days, and were used for western blotting to measure expression level of CD99. (T. thymocyte cell extract,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

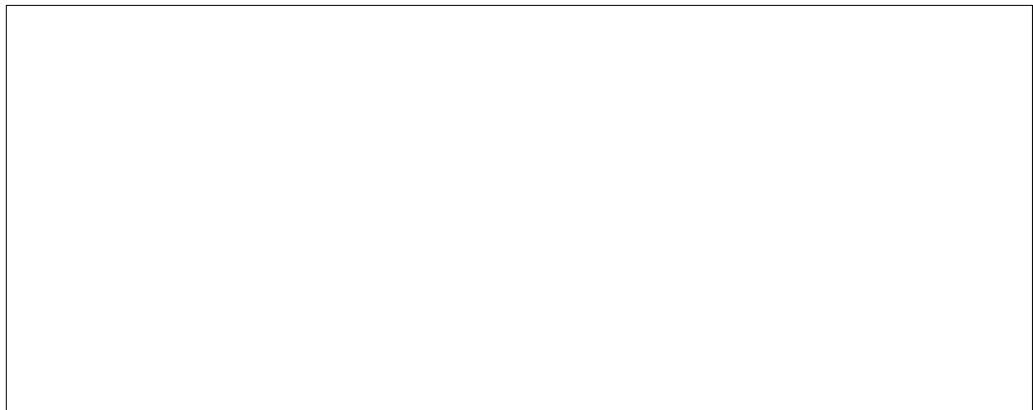


Fig. 2. Flow cytometric analysis of up-regulation of CD99 and MHC class I molecule in SH-SY5Y by treatment of TPA. (secondary antibody only, CD99 expression in DN16 antibody treated SH-SY5Y, MHC class I expression in W6/32 antibody treated SH-SY5Y).

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 promotor 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 promotor acitivity CD99 transient
transfection activity , CD99
promotor activity가 가 Northern
CD99 가 CD99 mRNA
가 . SH-SY5Y
CD99가 CD99 promoter
 . CD99 activity HeLa (data not

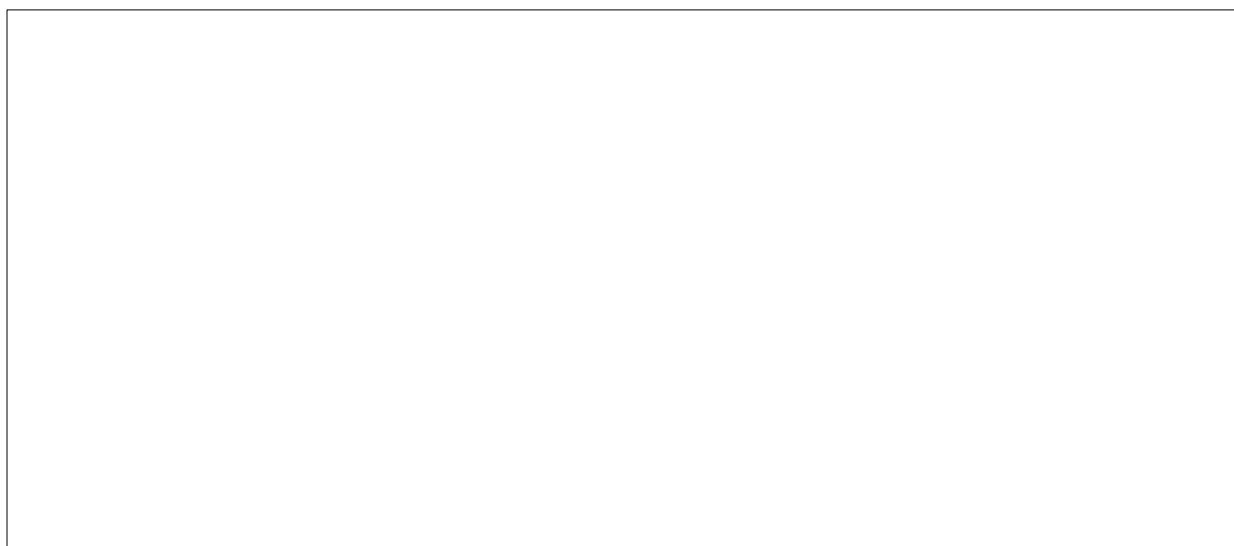


Fig. 3. Analysis of the CD99 expression in various cell lines. (A) Expression patterns of endogenous CD99 RNA. Total RNA was prepared from nonlymphoid (293, 293T, and HeLa) and lymphoid (BJAB, IM9, and Jurkat) cell lines, and 50 μ g of RNA from each cell line was separated by electrophoresis, and analyzed. 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).
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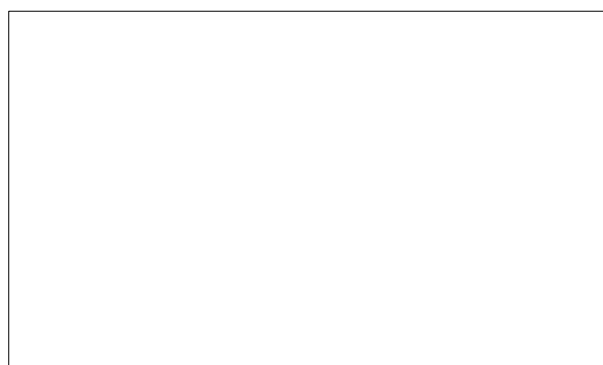


Fig. 4. Effect of retinoic acid and TPA on CD99 promoter activity in SH-SY5Y. SH-SY5Y were transiently transfected with p (-1654/+123). Substances were applied 1 day after transfection. Cells were collected for luciferase assay 48hrs post transfection. The solvent DMSO served as a control. Values represent 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 luciferase . TPA가 SH-SY5Y CD99 . SH-SY5Y , SH-N-SH , CD99 noradrenergic subclone 가 . SH-SY5Y 가 TPA (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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