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# Induction of clusterin/apoJ expression by histone deacetylase inhibitors in neural cells

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**Abstract**

The regulation of clusterin expression is poorly characterized although some regulatory elements have been identified, such as CpG-rich methylation domain. Environmental stress, aging, diet and diseases regulate DNA methylation and protein acetylation status but interestingly, the same insults increase clusterin expression in vivo. Our purpose was to elucidate whether histone deacetylase inhibitors, such as TSA, SAHA and M344, as well as an inhibitor of DNA methylation, 5′-aza-2′-deoxycytidine, could regulate the expression of clusterin in cultured neural cells. We observed that histone deacetylase inhibitors induced the expression of clusterin mRNA and protein in all neural cells studied. The induction of clusterin mRNA was blocked by actinomycin D which indicates that TSA regulates clusterin expression at the transcriptional level. An inhibitor of DNA methylation, 5′-aza-2′-deoxycytidine, itself did not affect the expression of clusterin mRNA but strongly potentiated the TSA-induced expression of clusterin. Proteasomal stress (MG-132 and PI-1 treatments) and apoptotic stress (okadaic acid treatment) did not affect clusterin expression which indicates that the induction of clusterin expression requires more specific inducers than cellular stress in general. Furthermore, LPS did not affect clusterin expression in N9 microglia although activated NF-κB signaling and IL-6 expression. CAPE and helenalin, inhibitors of NF-κB signalling, did not affect the clusterin mRNA expression either in non-treated or in TSA-treated N9 microglia. These observations suggest that clusterin induction is NF-κB-independent and unrelated to the inflammatory response in N9 microglia.


*Keywords:* Acetylation; Alzheimer; Epigenetics; HDAC; Inflammation; Neurodegeneration

## 1. Introduction

Clusterin is a heterodimeric glycoprotein identified over 20 years ago (Blaschuk et al., 1983). A single-copy clusterin gene encodes a 2 kb mRNA which directs the synthesis of a precursor protein of 449 amino acids (Wilson and Easterbrook-Smith, 2000; Jones and Jomary, 2002). The precursor protein is proteolytically cleaved into α- and β-chains which form a heterodimer with five disulfide bridges. The mature protein contains N-linked carbohydrate structures attached at six possible glycosylation sites. Clusterin is ubiquitously expressed in almost all mammalian tissues but its expression is limited to particular cell types, e.g. in brain in astrocytes and in some neuronal populations in cerebellum and hippocampus (Pasinetti et al., 1994). The function of clusterin has remained elusive although a role as a chaperone both inside and outside of cells, as well as in cholesterol transport and cell-interactions have been proposed (Rosenberg and Silkensen, 1995; Wilson and Easterbrook-Smith, 2000). Clusterin has several alternative names due to its different functions (Jones and Jomary,

*Abbreviations:* ApoE, apolipoprotein E; ApoJ, apolipoprotein J; 5-aza-dC, 5′-aza-2′-deoxycytidine; FBS, fetal bovine serum; CAPE, caffeic acid phenethyl ester; CLU, clusterin; EMSA, electrophoretic mobility shift assay; GRE, glucocorticoid/androgen response element; HDAC, histone deacetylase; IL-6, interleukin-6; LPS, lipopolysaccharide; M344, 4-dimethylamino-*N*-(6-hydroxycarbamoylhexyl)-benzamide; PNGase F, peptide-*N*-glycosidase F; PI-1, proteasomal inhibitor-1; SAHA, suberoylanilide hydroxamic acid; TSA, trichostatin A

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2002). For instance, clusterin forms a high density lipoprotein complex with apolipoprotein A-1 in human plasma, hence it is also called apolipoprotein J (apoJ) (Jenne et al., 1991).

There are many reports demonstrating that clusterin expression increases in association with aging, tissue injuries and in several diseases (Rosenberg and Silkensen, 1995; Trougakos and Gonos, 2002). In Alzheimer's disease, clusterin aggregates in senile plaques and cerebrovascular amyloid depositions (Kida et al., 1995; Verbeek et al., 1998). The role of clusterin in the amyloid plaque formation is still open although transgenic animal models indicate that apolipoprotein E and clusterin have a key role in the formation of amyloid plaques (Holtzman, 2004). The regulation of clusterin expression is not well characterized although the promoter has been cloned and some regulatory elements are known (Rosemblit and Chen, 1994; Wong et al., 1994; Michel et al., 1997). The clusterin promoter contains binding sites for Sp1, AP-1 and HSF1 transcription factors. Furthermore, the clusterin promoter contains CpG-rich methylation domain (Rosemblit and Chen, 1994; Wong et al., 1994) and thus clusterin expression might be regulated by DNA methylation and histone acetylation (Nakao, 2001). Interestingly, several environmental stresses, aging, diet and diseases regulate DNA methylation and protein acetylation status in cells (Deckert and Struhl, 2001; Timmermann et al., 2001; Chang and Min, 2002).

Several studies have shown that the inhibition of classes I and II histone deacetylases, e.g. with trichostatin A (TSA), induces a selective hyperacetylation of cellular proteins and changes in the gene expression in about 2% of genes (Yoshida et al., 1995; Marks et al., 2001; Johnstone, 2002). Our purpose was to elucidate whether histone deacetylase inhibitors, such as TSA, SAHA and M344, as well as an inhibitor of DNA methylation, 5′-aza-2′-deoxycytidine, could regulate the expression of clusterin in cultured neural cells. Our results revealed that the induction of clusterin mRNA and protein expression by histone deacetylase inhibitors is a general response and not cell-type specific among neural cells. An inhibitor of DNA methylation, 5′-aza-2′-deoxycytidine, itself did not affect the expression of clusterin mRNA but strongly potentiated the TSA-induced expression of clusterin. Furthermore, proteasomal stress and apoptotic stress did not affect clusterin expression which indicates that the induction of clusterin expression needs more specific inducers than cellular stress in general.

## 2. Materials and methods

### 2.1. Materials

Trichostatin A, CAPE (caffeic acid phenethyl ester) and 5′-aza-2′-deoxycytidine were purchased from Sigma (St. Louis, USA). SAHA (suberoylanilide hydroxamic acid) and M344 (4-dimethylamino-*N*-(6-hydroxycarbamoylhexyl)-benzamide) were obtained from Alexis Biochemicals (Lausen, Switzerland). Lipopolysaccharide used in all experiments was from *Escherichia coli* 055:B5 lyophilized powder (L 6529 from Sigma). Helenalin was from BIOMOL Research Labs (Plymouth Meeting, USA) and proteasome inhibitors, MG-132 and proteasome inhibitor 1 (PI-1), were from Calbiochem (Merck, Darmstadt, Germany).

### 2.2. Cell culture

The effect of histone deacetylase inhibitors was verified using different primary cells and continuous cell lines. Primary astrocytes were isolated from 1- to 2-day-old Wistar rat cerebral cortices and midbrain, isolated and cultured as we have described earlier (Suuronen et al., 2003). Hippocampal neurons were isolated from 17-day-old Wistar rat embryos. We have recently described the procedures utilized to isolate and culture rat hippocampal neurons (Suuronen et al., 2003).

The human neuroblastoma cells studied were IMR-32, SH-SY5Y and SK-N-AS and the mouse neuroblastoma was Neuro-2a. All these lines were obtained from American Type Culture Collection (ATCC) as well as rat C6 glioma cells. HN-10 neuroblastoma cells were originally established from rat hippocampus (Lee et al., 1990). We also studied the effect of histone deacetylase inhibitors on non-neuronal human ARPE-19 and MCF-7. All these lines were obtained from ATCC. All cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma, St Louis, USA) supplemented with 10% FBS (Sigma), 2 mM L-glutamine (Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco). ARPE-19 cell were cultured in the DMEM medium supplemented 1:1 with F-12 growth supplement (Gibco). Cells were plated on Nunc plastic dishes (Nalgene) at the density of (3–4) $\times 10^4$ cells/cm$^2$.

Murine microglial cell line N9 was kindly provided by Dr. Paola Ricciardi-Castagnoli (University of Milano-Bicocca, Milan, Italy). N9 cells were cultured in Iscove's Modified Dulbecco's Medium (Gibco) with 5% heat-inactivated fetal calf serum (Gibco) on Nunc (Nalgene) dishes and clusters. N9 microglia cells were plated at a density of $4 \times 10^4$ cells/cm$^2$ for 24 h to start the experiment.

### 2.3. Northern blot analysis

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. Four micrograms of total RNA was resolved in 1% agarose gel (Sigma) in MOPS buffer with 0.6 M formaldehyde. RNA was transferred to Magna Graph nylon membrane (Osmonics, Minnetonka, USA) by downward capillary process, fixed by UV irradiation at 72 mJ in UV Stratalinker 2400 (Stratagene, La Jolla, USA), dried under 55 °C for an hour, and stained with methylene blue to check the transfer efficiency. Gene-specific fragments for riboprobes were generated by PCR, cloned into pGEM-T Easy vector (Promega, Madison, USA), and verified by sequencing. The primer sequences used for the clusterin specific probe were: CLU-A, 5′-gcatcgactccctgctggag and CLU-B, 5′-cttcac(c/g)accacctcagtgacac. The clusterin probe was planned to be suitable to hybridize human (NM_001831), mouse (NM_013492) and rat (NM_012679) mRNAs. The primer sequences used for interleukin-6 were: IL6-A, 5′-gttgccttcttgggactgatg and IL6-B, 5′-tggatggtcttggtccttagc. The $^{32}$P-labelled riboprobes were generated with the Strip-EZ kit (Ambion, Austin, USA). Hybridization was performed in the modified high-stringency Church buffer as described earlier in detail (Helenius et al., 2001). After hybridization, the membranes were rinsed with 1× SSC, 0.2% SDS and