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## Valproic acid Induces Transcriptional Activation of Human CMP-NeuAc: Lactosylceramide α2,3-Sialyltransferase (hST3Gal V) Gene in Neuroblastoma Cells

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To investigate whether valproic acid (VPA) modulates human CMP-NeuAc : Lactosylceramide α2,3-Sialyltransferase (hST3Gal V) mRNA expression, as a part of ganglioside GM3 biosynthesis, in human neuroblastoma cells. Using RT-PCR and immunofluorescent confocal microscopy, we examined hST3Gal V mRNA and GM3 levels during VPA-induced differentiation of human neuroblastoma SK-N-BE(2)-C cells. We characterized the VPA-inducible promoter region within the hST3Gal V gene using luciferase constructs carrying 5′-deletions of the hST3Gal V promoter. RT-PCR indicated that VPA-mediated hST3Gal V induction is transcriptionally regulated. Functional analysis of the 5′-flanking region of the hST3Gal V gene demonstrated that the -177 to -83 region, which contains a cAMP-responsive element (CRE) at -143, functions as the VPA-inducible promoter by actively binding CRE binding protein (CREB). In addition, site-directed mutagenesis and EMSA indicated that the CRE at -143 is crucial for the VPA-induced expression of hST3Gal V in SK-N-BE(2)-C cells. Our results isolated the core promoter region in the hST3Gal V promoter, a CRE at -143, and demonstrated that it is essential for transcriptional activation of hST3Gal V in VPA-induced SK-N-BE(2)-C cells. Subsequent CREB binding to this CRE mediates VPA-dependent upregulation of hST3Gal V gene expression.

**Key words:** Valproic acid; hST3Gal V; human neuroblastoma; CREB; ranscriptional regulation

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## Isolation and Functional Analysis of the Glioblastoma-Specific Promoter Region of the Human GD3 Synthase Gene

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We identified the promoter region of the human GD3 synthase gene to elucidate the mechanism underlying the regulation of human GD3 synthase gene expression in human glioblastoma U-87MG cells. The 5′-rapid amplification of cDNA end (5′-RACE) using mRNA prepared from U-87MG cells revealed the presence of transcription start site of human GD3 synthase gene, and the 5′-end analysis of its product showed that transcription started from 646 nucleotides upstream of the translational initiation site. Promoter analyses of the 5′-flanking region of the human GD3 synthase gene using luciferase gene reporter system showed the strong promoter activity in U-87MG cells. Deletion study revealed that the region as the core promoter from -1308 to -1000(A of the translational start ATG as position +1) was indispensable for endogenous expression of human GD3 synthase gene. This region lacks apparent TATA and CAAT boxes, but contains putative binding sites for transcription factors AREB6 and E1K-1. Site-directed mutagenesis and transient transfection assays demonstrated that both AREB6 and E1K-1 elements in this region are required for the promoter activity in U-87MG cells. Further analysis by electrophoretic mobility shift assays using specific competitors revealed that both AREB6 and E1K-1 nuclear proteins specifically interacts with these element, respectively. Taken together, these results indicate that both AREB6 and E1K-1 may play an essential role in the transcriptional activity of human GD3 synthase gene essential for GD3 synthesis in U-87MG cells.

**Key words:** Promoter; human GD3 synthase; glioblastoma; transcription factor