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hST8Sia III gene expression via the PI-3K signal transduction pathway during KCl-induced differentiation of human glioblastoma U-87 cells

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We have shown that KCl known as an inducer for differentiation of neuronal cells increases the human Sia-α2,3-Gal-β1,4-GlcNAc-R:α2,8-sialyltransferase (hST8Sia III) gene transcription via phosphoinositide 3 kinase (PI-3K) in glioblastoma U-87MG cells. The induction of hST8Sia III by KCl is regulated at the transcriptional level in a dose- and time-dependent manner as evidenced by reverse transcription polymerase chain reaction (RT-PCR). To elucidate the mechanism underlying the regulation of hST8Sia III gene expression inU-87MG cells induced by KCl, we characterized the promoter region of the hST8Sia III gene. Functional analysis of the 5′-flanking region of the hST8Sia III gene by the transient expression method showed that the -1194 to -816 region functions as the KCl-inducible promoter in U-87MG cells. Furthermore, as evidenced by Western blot analysis and RT-PCR, KCl-induced expression of hST8Sia III gene was dependent on the PI-3K signal transduction pathway during the neuronal differentiation of U-87 cells, as an increase in -tubulin III known as a neuronal differentiation maker was observed. In KCl-depolarization on U-87 cells, the PI-3K-dependent promoter activation at the -1194 to -816 region upregulated expression of hST8Sia III gene. These results suggest that the expression of hST8Sia III gene via the PI-3K signaling pathway is enhanced during KCl-induced differentiation of U-87 cells by increasing expression of -tubulin III.

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NF-kB plays an essential role in the transcriptional activity of human GD3 synthase gene in Fas-activated T cell

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In this study the transcriptional regulation mechanisms involved in up-regulation of Fas-induced GD3 synthase gene have been investigated. To determine a Fas-activated promoter region of the human GD3 synthase gene, we cloned and characterized the 2.0 kb 5′-flanking region of the human GD3 synthase. The 5′-rapid amplification of cDNA end (5′-RACE) using mRNA prepared from Fas-induced Jurkat T cells revealed the presence of multiple transcription start sites of human GD3 synthase gene, and the 5′-end analysis of the longest its product showed that transcription started from 650 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 strong promoter activity in Fas-induced Jurkat T cells. Deletion study revealed that the region from 1146 to 646(A of the translational start ATG as position +1) was indispensable for the Fas response. This region lacks apparent TATA and CAAT boxes, but contains putative binding sites for transcription factors c-Ets-1, CREB, AP-1 and NF-kB. Base-substitution experiment showed that only NF-kB binding site of them is required for the maximal expression induced by Fas. Both DNase I footprint and electrophoretic mobility shift assays with the nuclear extract of Fas-induced Jurkat T cells revealed that NF-kB was bound specifically to the probe being mediated by its binding site in the promoter sequence. Taken together, these results indicate that NF-kB plays an essential role in the transcriptional activity of human GD3 synthase gene in Fas-induced Jurkat T cells. In addition, the translocation of NF-kB binding protein to nucleus by Fas-activation is also crucial for the increased expression of the GD3 synthase gene in Fas-activated Jurkat T cells.