[P01.017] Valproic Acid Effects on Histone Acetylation in the Survival Motor Neuron (SMN) Promoter

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OBJECTIVE: To characterize histone acetylation in the promoter region of the spinal muscular atrophy (SMA)-determining gene, *survival motor neuron* (*SMN*), and to describe the mechanism of action of valproic acid, a histone deacetylase inhibitor being considered for clinical trials in SMA. **BACKGROUND:** SMA is caused by deletion or mutation in the telomeric copy of the *SMN* gene. Embryonic lethality is rescued by the presence of a second centromeric copy of the gene, *SMN2*. Because severity of disease correlates with SMN protein deficiency, therapies have focused on increasing expression of the *SMN2* gene to restore normal protein levels. Histone deacetylase (HDAC) inhibitors, including valproic acid and phenylbutyrate, can modify gene expression and have been shown to increase SMN transcript and protein levels *in vitro*. The specific mechanism of action by which HDAC inhibitors increase SMN levels remains unknown, however. Furthermore, currently available HDAC inhibitors are nonspecific, inhibiting most HDAC enzymes, which may limit their tolerability. By characterizing the epigenetic controls of *SMN* gene expression, we hope to identify refined molecular targets for SMA therapy development. **DESIGN/METHODS:** Promoter activity experiments were performed in NSC-34 cells (mouse motor neuron-like cells) that had been stably transfected with a 3.4 kb fragment of the SMN2 promoter fused to the β-lactamase reporter gene. Chromatin immunoprecipitation assays were used to evaluate the association of acetylated histones and other proteins with the SMN promoter in NSC-34 cells and fibroblasts derived from type I SMA patients. During treatment, freshly prepared valproic acid was added to the cell media at a final concentration of 0.5-5 mM. **RESULTS:** SMN promoter activity increased in NSC-34 cells following treatment with valproic acid. A maximal increase of 2-fold was seen after treatment with 2 mM valproic acid for 24 hours. A time course experiment indicated no increase in promoter activity before 18 hours. Chromatin immunoprecipitation experiments showed an increase in acetylated histones associated with the SMN promoter that was first evident after 4 hours of treatment. At baseline, histone acetylation within the endogenous SMN promoter in fibroblasts derived from SMA patients is high in downstream regions near the transcription initiation site and low in upstream regions. Following treatment with VPA, levels of acetylated histones increase in all regions of the SMN promoter with the greatest differences evident in upstream regions. **CONCLUSIONS:** Valproic acid increases SMN promoter activity. This effect is preceded by an increase in acetylated histones associated with all regions of the SMN promoter. Further efforts are now being undertaken to identify the specific HDAC enzymes that modify SMN gene expression.

Category - Neurogenetics and Gene Therapy

SubCategory - Therapeutics