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ORIGINAL ARTICLE

Transcript, methylation and molecular docking analyses of the effects of HDAC inhibitors, SAHA and Dacinostat, on *SMN2* expression in fibroblasts of SMA patients

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Several histone deacetylase inhibitors (HDACis) are known to increase Survival Motor Neuron 2 (SMN2) expression for the therapy of spinal muscular atrophy (SMA). We aimed to compare the effects of suberoylanilide hydroxamic acid (SAHA) and Dacinostat, a novel HDACi, on SMN2 expression and to elucidate their acetylation effects on the methylation of the SMN2. Cell-based assays using type I and type II SMA fibroblasts examined changes in transcript expressions, methylation levels and protein expressions. *In silico* methods analyzed the intermolecular interactions between each compound and HDAC2/HDAC7. SMN2 mRNA transcript levels and SMN protein levels showed notable increases in both cell types, except for Dacinostat exposure on type II cells. However, combined compound exposures showed less pronounced increase in SMN2 transcript and SMN protein level. Acetylation effects of SAHA and Dacinostat promoted demethylation of the SMN2 promoter. The *in silico* analyses revealed identical binding sites for both compounds in HDACs, which could explain the limited effects of the combined exposure. With the exception on the effect of Dacinostat in Type II cells, we have shown that SAHA and Dacinostat increased SMN2 transcript and protein levels and promoted demethylation of the *SMN2* gene.
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INTRODUCTION

With an incidence of 1/6000 to 1/10 000 live births and a carrier frequency of 1/40 to 1/50, spinal muscular atrophy (SMA) is the second most common cause of an autosomal recessive hereditary disorder after cystic fibrosis.[1] *Survival Motor Neuron 1* (*SMN1*) gene deletion has been detected in 96% of SMA patients, and the remaining patients showed intragenic mutations of the gene. *Survival Motor Neuron 2* (*SMN2*) is a highly homologous copy of *SMN1*.[1,2] Affected patients present variable copy numbers of *SMN2* that are inversely related to SMA severity.[3,4]

Enhancing *SMN2* gene expression using small-molecule compounds has been proposed as a therapeutic strategy for treating SMA.[5] Several histone deacetylase inhibitors (HDACis), such as short chain fatty acids (valproic acid (VPA), phenylbutyrate), hydroxamic acids (LBH589 (Panobinostat), suberoylanilide hydroxamic acid (SAHA), trichostatin A) and benzamides (M344 (*N*-hydroxyl-7-aminoheptanamide), MS-275), have shown promising therapeutic effects on SMA-derived cells (reviewed in Mohseni *et al.*[6]).

Histone acetyltransferases relax chromatin by adding acetyl groups, whereas histone deacetylases (HDACs) neutralize the actions of histone acetyltransferases by removing acetyl groups. HDACis increase gene accessibility to transcriptional machinery by preventing deacetylation, thus maintaining histone acetylation and subsequently activating gene promoters.[7,8]

Dacinostat is a new hydroxamate-based HDACi with potential anticancer activity. Dacinostat is a potent HDACi that is currently in a phase I clinical trial for the treatment of leukemia. Dacinostat is generally more potent than SAHA, another hydroxamic acid, in low nanomolar doses.[9] Dacinostat also shows fewer toxic effects to normal human hematopoietic cells.[10]

Other HDACis have been shown to increase SMN2 transcript by activating the *SMN2* promoter. SAHA, an HDACi, increased full-length SMN2 transcript levels in SMA.[8,11,12] However, there has been no report on the effect of Dacinostat on *SMN2* gene expression. In this study, we compared the effects of SAHA and Dacinostat on *SMN2* expression.

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