Modulation of Histone Deacetylases (HDACs) Expression in Patients with and without Systemic Lupus Erythematosus: Possible Drug Targets for Treatment

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Abstract
There is increasing evidence that epigenetic factors may play a role in the pathogenesis of Systemic Lupus Erythematosus (SLE). Both global and gene specific methylation is known to occur in lupus patients, as well as, changes in histone acetylation status. Histone acetylation is associated with active chromatin or activation of genes, whereas histone deacetylase (HDAC) activity is associated with silencing of genes. Therefore, HDACs have been targeted as potential therapeutic targets for a number of diseases, including lupus. The purpose of this study was to determine histone deacetylase (HDAC) expression in patients who are diagnosed with SLE compared to age-matched healthy controls. Quantitative real-time PCR expression levels of HDAC 1, HDAC 2 and HDAC 7 were investigated in peripheral blood mononuclear cells of African American and European American women. Our results showed that HDAC 1 expression is significantly (p < 0.0039) elevated in lupus patients compared to controls. HDAC 2 expression is also increased in lupus patients (p < 0.0427). However, HDAC 7 showed no significant difference (p < 0.4644) in expression in our SLE patients compared to their controls. Those lupus patients with a SLE disease activity index (SLEDAI) of 4 or greater showed lower expression of HDAC 1 (p < 0.0026) compared to those with modest disease and a SLEDAI of less than 4. However, in those lupus patients with a SLE disease activity index (SLEDAI) of 4 or greater showed increased expression of HDAC2 (p < 0.053) when compared to those with a SLEDAI of less than 4. This observation was also noted in HDAC7. Increased expression in HDAC 1 and 2 has been associated with induced kidney injury and induction of proinflammatory cytokines.

Keywords
Histone deacetylases, Systemic lupus erythematosus, Epigenetic regulation

Introduction
The difficulties in designing an effective pharmacological therapy for Systemic Lupus Erythematosus (SLE) are due in part to the complexities of its pathophysiology. However, recently researchers have begun to look at the role chromatin modification plays in SLE. Chromatin modification is important in the regulation of genomic expression. One of the essential parts of chromatin structure are the histones. Histones are responsible for binding the nucleosome and provide the entry and exit sites to DNA. Like DNA, histones can also be subjected to epigenetic events. A group of enzymes that have shown to play a key role in histone modification are histone deacetylases (HDACs). HDAC enzymes work on the amino terminal tail of histones. They are able to regulate gene expression by modulating histone acetylation patterns [1]. There are 18 genes identified as HDACs. These 18 genes can further be grouped into four classes based on their structural functional capabilities. There has been increasing evidence that targeting certain classes of HDACs can provide therapeutic benefit to patients with SLE. For example, preclinical studies have shown that using the HDAC inhibitor (HDACi), Trichostatin A (TSA), can reduce anti-DNA autoantibody production.

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HDAC 1, HDAC 2, and HDAC 7 mRNA expression was conducted using a Bio-Rad IQ5 quantitative Real Time Polymerase Chain Reaction Detection System (BIO-RAD, Hercules, CA). GAPDH was used as a housekeeping gene and as an endogenous control. qRT-PCR conditions were as follows: 50 °C for 2 minutes, 95 °C for 10 minutes (95 °C for 10 seconds, 56 °C for 45 seconds, 72 °C for 30 seconds) × 30 cycles. Relative quantitation’s of HDAC 1, HDAC 2 and HDAC 7 mRNA expressions were normalized to GADPH and fold changes were calculated using a 2^(-ΔΔCT) method. Primers utilized for both the histone deacetylases and GAPDH are listed in Table 1.

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<tr>
<th>Primers</th>
<th>Sense</th>
<th>Antisense</th>
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<tr>
<td>HDAC 1</td>
<td>GGAATCTATCGGCTTCCACA</td>
<td>AACAGGCCATGAATCTGGG</td>
</tr>
<tr>
<td>HDAC 2</td>
<td>5’GTGCCTCAGTTGCTTCATCA</td>
<td>GATGCAAGTGCGACAGATCA</td>
</tr>
<tr>
<td>HDAC 7</td>
<td>CCCAGCAAAACCTTCTACCAA</td>
<td>AAGCAGCCAGTGCTCAGGA</td>
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Statistical analysis

Statistical analyses were performed using GraphPad Prism Software Version 6.0 (San Diego, CA). A t-test were used for statistically significance. P < 0.05 was determined to be significant.

Results

In Figure 1 we compare mRNA expression of HDAC 1 from normal controls and lupus patients. Individual differences were noted among the patients. In addition, statistically significant differences in HDAC1 mRNA expression were observed between lupus compared to non-lupus. Furthermore, HDAC 1 mRNA expression levels were significantly higher (p < 0.0039) in patients with SLE compared to controls. In Figure 2, we determined the mRNA expression levels of HDAC 2 from normal and lupus patients. Our analysis indicated that HDAC 2 expression levels was significantly higher (p < 0.0427) in our SLE patients compared to our controls. However, HDAC 7 mRNA expression levels among SLE and control...
In the present study, we were interested in determining if HDAC expression was altered in Lupus patients as compared to non-Lupus patients. Our results demonstrated that the mRNA expression levels of Class I HDACs among SLE patients compared to controls were significantly different. Specifically, HDAC 1 and 2 were significantly up-regulated in SLE patients compared to controls. However, HDAC 7, which is a member of the class II HDACs, did not show a significant difference in expression level between SLE and controls patients. Of the histone deacetylases studied, HDAC 1 had significantly patients were not significantly different (Figure 3 and Figure 4) (p < 0.4644). Our results provide evidence that histone deacetylases may be involved in the pathogenesis of SLE and Class I HDACs should be further investigated as potential therapeutic targets. Furthermore, these results demonstrated that an increase in HDAC 2 (p < 0.053) and 7 (p < 0.0259) expression were observed more in lupus patients with a SLE disease activity index (SLEDAI) of 4 or higher, when compared to patients with more modest disease, Figure 5 and Figure 6. However, a decrease in expression of HDAC 1 (p < 0.0026) was noted in lupus patients with higher SLEDAIs.

Discussion

Histone deacetylases (HDACs) are critical for the maintenance of gene and chromosome silencing. Furthermore, HDACs assist in chromatin modification and transcriptional regulation of an organism’s genome. In the present study, we were interested in determining if HDAC expression was altered in Lupus patients as compared to non-Lupus patients. Our results demonstrated that the mRNA expression levels of Class I HDACs among SLE patients compared to controls were significantly different. Specifically, HDAC 1 and 2 were significantly up-regulated in SLE patients compared to controls. However, HDAC 7, which is a member of the class II HDACs, did not show a significant difference in expression level between SLE and controls patients. Of the histone deacetylases studied, HDAC 1 had significantly
higher mRNA expression than HDAC 2 and 7. There is increasing evidence that over expression of HDAC 1 is linked to various malignancies such as cancer and kidney damage [9]. In addition, studies have shown that an increase in HDAC 1 expression is linked to decrease survival rates [10,11]. This suggests that HDAC 1 expression could be a useful biomarker for disease progression in SLE patients. Furthermore, HDAC 1 may be a potential therapeutic target for pharmacological design. On the other hand, HDAC 2 showed a significant difference in expression between SLE and control patients however, this difference was not as significant as HDAC 1 expression. Researchers have provided evidence that HDAC 2 expression levels can be linked as a possible biomarker for survival; especially in cases of oral cancer [12]. However, in cancer models, HDAC 2 has been shown to play an anti-apoptotic role [13]. With one of the hallmarks of SLE being apoptotic complications, HDAC 2 targeting could prove a potential avenue for therapeutic analysis. This is further underscored by the fact that this study demonstrated an increase in expression of HDAC 2 in the SLE population. This suggests that HDAC 2 should be further investigated in SLE.

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Disclaimer

The findings and results reported in this manuscript are those of the authors and do not necessary represent the views of the US Food and Drug Administration.

References