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.

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 patients were not significantly different.

Patients and Methods

Study populations

Patients participating in this study were part of the LUPUS study at the Brody School of Medicine at East Carolina University. This study consisted of a total of 224 participants. The participants representing this study were ninety-six women diagnosed as having SLE based on SLE disease activity index (SLEDAI) scores and by anti-dsDNA antibody analysis and ninety-one controls that were age, sex, and ethnicity matched. No males were analyzed in this present study. Informed consent was obtained from all of our participants for blood samples to conduct our analysis. Also, this project was granted IRB approval from both The US Food and Drug Administration and from East Carolina Brody School of Medicine.

Blood collection and isolation of PBMC

For our analysis, blood samples were collected by venipuncture of the antecubital vein between 9:00 AM-12:00 PM. In order to maintain a similar circadian pattern between our participants with SLE and their matched control participants, collections were conducted at the same time of day and the same day of the week. Peripheral blood mononuclear cells were isolated from whole blood using PAXgene RNA Blood tubes at East Carolina University in Greenville, NC, placed in dry ice and stored at -80 °C until shipped to the National Center for Toxicological Research for analysis.

RNA isolation and quantitative real time PCR

RNA was extracted from peripheral blood mononuclear cells (PBMC) using a PAXgene RNA kit (QIAGEN, Valencia, CA). After extraction, all samples were tested for RNA integrity and concentration using a Bio-Rad Experion Automated Electrophoresis System (BIO-RAD, Hercules, CA). cDNA was created from RNA extractions using a Clontech Advantage® RT-for-PCR Kit (Clontech, Mountain View, CA).

Table 1: Primers used for Quantitative Real Time Polymerase Chain Reaction.

| Primers | Sense | Antisense |
|---------|-------|-----------|
| HDAC 1  | GGAATCTATCGGCCCTCACA | AACAGGCCATGAATCTGG |
| HDAC 2  | 5’GTGCCTCAGTTGCTCATCA | GATGCAGTGAGCCAAGATCA |
| HDAC 7  | CCCAGCAACCTTCTACCA | AAGCAGCCAGTACTCGGA |

Figure 1: Note: HDAC 1 mRNA expression among women with SLE compared to age-matched controls. GAPDH was used as the housekeeping gene for comparison. The p-value was (p < 0.0039) which indicated a significant difference between the two populations.
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
 Disclaimer

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

 References

1. Marks PA, Miller T, Richon VM (2003) Histone deacetylases. Curr Opin Pharmacol 3: 344-351.
2. Lu ZP, Ju ZL, Shi GY, Zhang JW, Sun J (2005) Histone deacetylase inhibitor Trichostatin A reduces anti-DNA autoantibody production and represses IgH gene transcription. Biochem Biophys Res Commun 330: 204-209.
3. Reilly CM, Thomas M, Gogal R Jr, Olgun S, Santo A, et al. (2008) The histone deacetylase inhibitor trichostatin A upregulates regulatory T cells and modulates autoimmunity in NZB/W F1 mice. J Autoimmun 31: 123-130.
4. Mishra N, Reilly CM, Brown DR, Ruiz P, Gilkeson GS (2003) Histone deacetylase inhibitors modulate renal disease in the MRL-lpr/lpr mouse. J Clin Invest 111: 539-552.
5. Shuttleworth SJ, Bailey SG, Townsend PA (2010) Histone Deacetylase inhibitors: new promise in the treatment of immune and inflammatory diseases. Curr Drug Targets 11: 1430-1438.
6. Szyf M (2010) Epigenetic Therapeutics in Autoimmune Disease. Clin Rev Allergy Immunol 39: 62-77.
7. Suh HS, Choi S, Khattar P, Choi N, Lee SC (2010) Histone Deacetylase Inhibitors Suppress the Expression of Inflammatory and Innate Immune Response Genes in Human Microglia and Astrocytes. J Neuroimmune Pharmacol 5: 521-532.
8. Garcia BA, Busby SA, Shabanowitz J, Hunt DF, Mishra N (2005) Resetting the Epigenetic Histone Code in the MRL-lpr/lpr Mouse Model of Lupus by Histone Deacetylase Inhibition. J Proteome Res 4: 2032-2042.
9. Sudo T, Mimori K, Nishida N, Kogo R, Iwaya T, et al. (2011) Histone deacetylase 1 expression in gastric cancer. Oncol Rep 26: 777-782.
10. Minamiya Y, Ono T, Saito H, Takahashi N, Ito M, et al. (2011) Expression of histone deacetylase 1 correlates with a poor prognosis in patients with adenocarcinoma of the lung. Lung Cancer 74: 300-304.
11. Theocharis S, Klijianienko J, Giaginis C, Rodriguez J, Jouflroy T, et al. (2011) Histone deacetylase-1 and -2 expression in mobile tongue squamous cell carcinoma: associations with clinicopathological parameters and patients survival. J Oral Pathol Med 40: 706-714.
12. Chang HH, Chiang CP, Hung HC, Lin CY, Deng YT, et al. (2009) Histone deacetylase 2 expression predicts poorer prognosis in oral cancer patients. Oral Oncol 45: 610-614.
13. Jurkin J, Zupkovitz G, Lagger S, Grausenburger R, Hagelkruys A, et al. (2011) Distinct and redundant functions of histone deacetylases HDAC1 and HDAC2 in proliferation and tumorigenesis. Cell Cycle 10: 406-412.

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 decreased 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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