ABSTRACT NUMBER: 3132

Histone Deacetylase 5 Is Overexpressed in Scleroderma Endothelial Cells and Impairs Angiogenesis Via Repressing Pro-Angiogenic Factors

Pei-Suen Tsou[1,2], M. Asif Amin[3], Elena Schiopu[2,4], David A. Fox[3], Dinesh Khanna[1,2] and Amr H. Sawalha[1], [1]Division of Rheumatology, University of Michigan, Ann Arbor, MI, [2]University of Michigan Scleroderma Program, Ann Arbor, MI, [3]Internal Medicine, Division of Rheumatology, University of Michigan Medical Center, Ann Arbor, MI, [4]Internal Medicine, Division of Rheumatology, University of Michigan, Ann Arbor, MI

Meeting: 2015 ACR/ARHP Annual Meeting

Date of first publication: September 29, 2015

Keywords: Angiogenesis, epigenetics and histone acetylation, Scleredema

SESSION INFORMATION

Date: Tuesday, November 10, 2015

Session Title: Systemic Sclerosis, Fibrosing Syndromes and Raynaud's - Pathogenesis, Animal Models and Genetics II

Session Type: ACR Concurrent Abstract Session

Session Time: 2:30PM-4:00PM

Background/Purpose:

Scleroderma (SSc) is a complex disease characterized by inflammation, vascular complications, and excessive deposition of extracellular matrix. Vascular dysfunction represents a disease initiating event in SSc. Indeed, endothelial cell (EC) damage is thought to trigger a self-fueling process that results in tissue fibrosis. Recent data suggest that epigenetic dysregulation impairs normal angiogenesis and can result in abnormal blood vessel growth patterns in various disease conditions. Studies have shown that histone deacetylases (HDACs) control EC proliferation and participate in EC migration. Specifically, HDAC5 appears to be anti-angiogenic through the repression of pro-angiogenic factors such as basic fibroblasts growth factors (bFGF), vascular endothelial growth factor (VEGF), and epherin B2. The phenotypic and functional abnormalities of SSc ECs are stable *in vitro*over multiple generations of tissue culture, suggesting that persistent epigenetic changes might play a role in EC dysfunction in SSc. We hypothesized that HDAC5 contributes to impaired angiogenesis in SSc by repressing pro-angiogenic factors in ECs.

Methods:

Dermal ECs were isolated from biopsies from patients with diffuse cutaneous SSc. HDAC5, VEGF, and ephrin B2 expression were determined by qPCR. HDAC5 was knocked down using HDAC5 siRNA. Angiogenesis was assessed by an *in vitro*Matrigel tube formation assay. bFGF and VEGF in culture media were measured using ELISA. A paired t-test was used to compare differences between groups, and a p-value of <0.05 was considered significant. An assay for transposase-accessible chromatin using sequencing (ATAC-seq) was performed to assess and localize genome-wide effects of HDAC5 knockdown on chromatin accessibility.

Results:

The expression of HDAC5 was significantly increased in SSc ECs compared to normal ECs (0.0058 ± 0.0017 vs. 0.0028 ± 0.0003, $p<0.05$) while pro-angiogenic ephrin B2 was down regulated (0.0021 ± 0.0011 vs. 0.0096 ± 0.0005, $p<0.05$). Cells transfected with HDAC5 siRNA showed 87% knockdown in SSc ECs. Silencing of HDAC5 in SSc ECs restored normal angiogenesis, as reflected by a significant increase in tube formation on Matrigel compared to sham-transfected cells. After HDAC5 knockdown, ephrin B2 mRNA decreased 50% compared to sham-transfected group in SSc ECs. In contrast, SSc ECs released increased amounts of VEGF and bFGF into the cell culture media (2 and 1.2 fold for VEGF and bFGF). In addition, VEGF mRNA increased significantly after HDAC5 knockdown (2.9 fold, $p<0.05$). ATAC-seq was used to identify additional HDAC5-regulated targets in EC, which will help to further mechanistically understand the anti-angiogenic effects of HDAC5.

Conclusion:

By knocking down HDAC5 in SSc ECs, we were able to restore the tube-forming ability of these cells. Our data indicate that overexpression of HDAC5 in SSc gears ECs to an anti-angiogenic state via repressing pro-angiogenic factors such as VEGF and bFGF. This appears to be a complicated process, as HDAC5 knockdown decreased pro-angiogenic ephrin B2. We provided a link between epigenetic regulation and impaired angiogenesis in SSc, and present a novel mechanism for the dysregulated angiogenesis that characterizes this disease.

Disclosure: P. S. Tsou, None; **M. A. Amin**, None; **E. Schiopu**, None; **D. A. Fox**, None; **D. Khanna**, Bristol-Myers Squibb, 2,EMD Serono, 2,Genentech and Biogen IDEC Inc., 2,Bayer, 5,Biogen Idec, 5,Cytori, 5,EMD Serono, 5,Forward, 5,Genentech and Biogen IDEC Inc., 5,Gilead, 5,Lycera, 5,Seattle Genetics, 5; **A. H. Sawalha**, None.