Regulation of inflammation by histone deacetylases in rheumatoid arthritis: beyond epigenetics
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CHAPTER 1

SCOPE OF THIS THESIS
The studies presented in this thesis were undertaken to characterize how histone deacetylase (HDAC) activity regulates inflammatory responses in rheumatoid arthritis (RA) synovial tissue, to investigate the consequences of inhibiting HDAC activity in RA synovial cells, and to identify molecular mechanisms underlying modulation of cellular inflammatory activation by HDAC inhibitors (HDACi).

Post-translational modification of proteins by reversible acetylation is catalyzed by two classes of enzymes, histone acetyl transferases (HATs) and HDACs and is one of the central mechanisms responsible for maintaining cell homeostasis and regulating cellular responses to environmental stimuli. While reversible acetylation of histones is an essential process in the epigenetic regulation of gene expression, some 1700 non-histone proteins, including components of intracellular signaling networks, transcription factors and structural proteins can also be directly regulated by reversible acetylation. Reduced activity and expression of HDACs contribute to pathology in inflammatory lung diseases, such as asthma and chronic obstructive pulmonary disease (COPD), and compounds restoring HDAC activity normalize inflammatory responses of alveolar macrophages and have demonstrated initial clinical efficacy. At the initiation of the research encompassed in this thesis, conflicting observations emerged regarding whether similar changes in HDAC activity and expression might contribute to pathology in RA. In chapter 2 we summarize the current knowledge about the contributions of aberrant epigenetic regulation, namely DNA methylation, post-translational histone modifications and aberrant microRNA expression, to the pathology of RA. Next, in chapter 3 we review recent studies analyzing therapeutic potential of targeting HDAC activity in RA and asthma as examples of chronic immune-mediated inflammatory diseases. In chapter 4 we examine whether reported alterations in synovial HDAC activity in RA are associated with changes in cellular protein acetylation, and analyze the relationship of protein acetylation status and HDAC family member mRNA expression with clinical parameters of disease activity as well as local expression of inflammatory mediators. We also assess how stimulation with inflammatory cytokines modulates HDAC expression in RA fibroblast-like synoviocytes (FLS).

Several previous reports indicated that HDACi suppress production of inflammatory cytokines by immune cells and are potent therapeutics in animal models of chronic and acute inflammatory disorders, including arthritis. However, little is known about HDACi regulation of RA synovial cell inflammatory activation, and early reports suggested that RA synovial cells, especially macrophages, might be resistant to anti-inflammatory effects of HDACi due to aberrant HDAC expression. In chapter 5 we compare HDACi effects on cytokine production and viability of macrophages derived from healthy donor peripheral blood and RA patient synovial fluid, and explore the global effects of HDACi treatment on the secretion of cytokines, chemokines and angiogenic factors by RA synovial biopsy explants.

Although anti-inflammatory properties of HDACi are well characterized, the mechanism(s) underlying HDACi-mediated suppression of cytokine production remain poorly understood. Gene array studies of cells treated with HDACi reporting that only a small proportion of expressed genes are modulated by HDACi, similar numbers of which are upregulated and downregulated, argue against a model in which HDACi-induced histone hyperacetylation leads to ultimate induction of gene expression, and suggest the involvement that non-epigenetic mechanisms, such as regulation of transcription factor activity or posttranscriptional events. In chapter 6 we perform detailed analyses of HDACi effects on early signaling events triggered by inflammatory stimulation. In the rheumatoid joint not only macrophages, but also stromal FLS produce large quantities of cytokines, including IL-6, which is a key mediator...
of RA pathology.\textsuperscript{14,15} We therefore examine whether HDACi modulate production of IL-6 by RA FLS, and test whether HDACi modulate the phosphorylation status of mitogen-activated protein kinases (MAPKs), activation of the activator protein-1 (AP-1) pathway components, and DNA binding as well as nuclear retention of nuclear factor-κB (NF-κB) subunits, all of which have been reported to be modulated by reversible acetylation.\textsuperscript{16} Finally, we investigate the influence of HDACi on posttranscriptional regulation of IL-6 gene expression by changes in mRNA stability. In chapter 7 we use quantitative PCR array systems to identify genes involved in inflammatory responses, cell survival, angiogenesis, regulation of extracellular matrix and adhesion, expression of which is modulated by HDACi through acceleration of mRNA degradation, and make use of bioinformatic tools to identify shared mechanisms by which HDACi may regulate expression of these genes in FLS.

In chapter 8 we extend our analyses of HDACi regulation of transcription factor activity to forkhead box O (FoxO) family members. In light of previous reports suggesting that inactivation and reduced expression of FoxO proteins contributes to inflammation in RA,\textsuperscript{17} we analyze mRNA expression profiles of FoxO1, FoxO3a and FoxO4 in FLS derived from RA and OA patients, and test if FLS exposure to HDACi restores FoxO DNA binding activity and mRNA accumulation. Finally, we use adenoviral overexpression systems to explore the biological consequences of constitutive FoxO1 activation in RA FLS.

REFERENCES

7. Ospelt C, Reedquist KA, Gay S, Tak PP. Inflammatory memories: is epigenetics the missing link to persistent stromal cell activation in rheumatoid arthritis? Autoimmun Rev 2011; 10(9):519-524.