Molecular mechanisms of histone deacetylases in rheumatoid arthritis fibroblast-like synoviocytes

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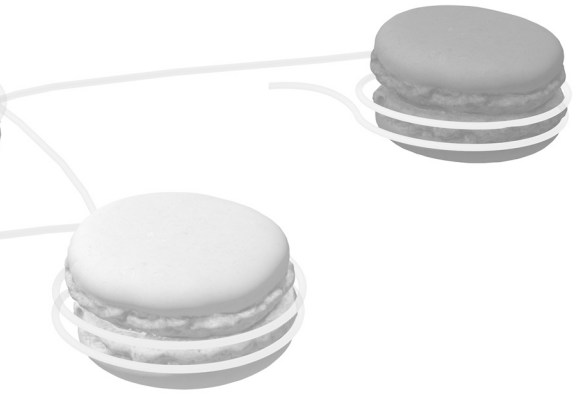

Chapter 1

PURPOSE OF THE THESIS

The studies presented in this thesis were designed with the intention to characterize the expression and activity of class I and class II histone deacetylases (HDACs) in rheumatoid arthritis (RA) synovial tissue and fibroblasts-like synoviocytes (FLS), and to identify the molecular and anti-inflammatory mechanisms of action of broad- and isoform-selective HDAC inhibitors (HDACi).

Histones modifying enzymes comprise a class of epigenetic regulators that, by controlling chromatin accessibility and transcriptional initiation, insure correct cellular and inflammatory responses. Among these, histone acetyltransferases (HAT) mediate the transfer of acetyl groups from acetyl coenzyme A (acetyl-CoA) to the ε-amino groups of lysine residues, while HDACs counterbalance HAT activity by deacetylating histones and non-histone proteins.[1] The balance between HAT and HDAC activity has often been found deregulated in pathological conditions, including RA and other forms of chronic inflammatory diseases. Specifically, at the initiation of this thesis research, increased HDAC activity and expression were reported in cells and tissue from RA patients, and associated with the production of synovial inflammatory mediators.[2-4]

In **chapter 2** we review the current knowledge on the acetylome signature in RA and in other rheumatic diseases, both in terms of epigenetic control of histone acetylation, and in a broader scope, of non-histone protein acetylation. We describe the mechanisms of actions of histone remodeling enzymes and their possible association with pathological manifestations of rheumatic diseases, and summarize recent studies reporting the therapeutic potential of HDAC inhibitors in RA models. In **chapter 3** we evaluate the expression of different HDAC isoforms in RA synovial tissue, and the relationship between HDAC expression and the expression of synovial pro-inflammatory factors and parameters of RA disease activity. We further explore histone and global lysine acetylation levels in RA, osteoarthritis (OA) and psoriatic arthritis (PsA) synovial tissues, with the aim to understand the relationship between synovial HDAC expression and synovial acetylation. Finally, we assess how diverse inflammatory triggers influence HDAC expression and activity in RA FLS, and how changes in HDAC function affect cellular inflammatory activation.

Several reports indicated that HDACi act as potent suppressors of inflammation *in vitro*, and display anti-rheumatic properties *in vivo*.[5,6] Moreover, recent studies showed predominant associations between class I HDACs and RA disease activity, and promising therapeutic benefits of selective class I HDAC inhibitors.[7-9] In **chapter 4**, we therefore investigate whether selective targeting of HDAC1/2, HDAC8 and HDAC3, by the use of inhibitors or knockdown strategies, displays similar anti-inflammatory features compared to the pan-HDACi ITF2357. We describe how HDAC depletion affects downstream signaling pathwaysin RA FLS, and how it ultimately controls inflammatory responses.

In **chapter 5** we elucidate the molecular mechanisms by which HDACi exert their anti-inflammatory and immunomodulatory properties. Specifically, we investigate whether, besides their documented effects on transcriptional regulation, HDACi can also act on the post-translational control of cytokine mRNA stability, a phenomenon previously described.[5] We further evaluate the possible involvement of microRNAs (miRNAs) and AU-rich element binding proteins (ARE-BPs) in the HDACi-mediated regulation of mRNA decay. In **chapter 6** we extend our studies on mRNA stability to Forkhead box

protein O1 (FoxO1), one pro-apoptotic and stress-control factor whose expression is induced by HDACi, but not regulated at the level of mRNA stability. We investigate whether other signaling pathways can regulate FoxO1 mRNA decay, such as those of phosphatidylinositide 3 kinase - protein kinase B (PI3K–PKB) and the mitogen-activated protein kinases (MAPKs). We additionally analyze the expression of FoxO family members (FoxO1, FoxO3a and FoxO4) in samples derived from RA and OA patients, and address the functional role of FoxO1 in RA FLS by using overexpression and knockdown techniques.

Taken together, the studies presented in this thesis aim to contribute to the understanding of the complex molecular mechanisms through which HDACs regulate inflammation, and to provide rationale for the development and use of isoform-selective HDACi in RA.

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