The role of histone deacetylases in rheumatoid arthritis fibroblast-like synoviocytes

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Abstract
RA (rheumatoid arthritis) is an inflammatory disease of synovial joints affecting approximately 1% of the population. One of the main cell types involved in damage to RA joint tissue is the FLSs (fibroblast-like synoviocytes). These have a semi-transformed, auto-aggressive phenotype typified by loss of contact inhibition, reduced apoptosis and the production of matrix-degrading enzymes. The mechanisms involved in the development of this phenotype are unclear; however, increasing evidence implicates alterations in the epigenetic regulation of gene expression. Reduced acetylation of amino acids in the tails of histone proteins is an epigenetic mark associated with transcriptional repression and is controlled by the HDAC (histone deacetylase) enzyme family. To date, evidence has implicated HDACs in the auto-aggressive phenotype of FLSs, and administration of HDAC inhibitors to both animal models of RA and individuals with juvenile arthritis has shown efficacy in attenuating inflammation and tissue damage. This highlights a role for HDACs in disease pathogenesis and, more importantly, that HDACs are potential novel therapeutic targets.

RA (rheumatoid arthritis)
RA is a chronic autoimmune inflammatory disease of synovial joints. It has a prevalence of approximately 1% in Europe and the U.S.A., and is three times more common in females [1]. It is a heterogeneous condition, but can broadly be stratified by the presence or absence of anti-cyclic citrullinated peptide antibodies [2]. The majority of RA patients are treated with drugs such as methotrexate that act to modulate immune activity and reduce cartilage and bone damage. Over the last decade, the use of biological therapies targeting pro-inflammatory cytokines such as TNF (tumour necrosis factor) and IL (interleukin)-6, B-cells or other immune cell interactions have revolutionized outcomes for patients with RA. These treatments, however, are expensive, require both long-term and systemic administration (rather than oral administration), and are ineffective in a significant proportion of patients [3]. There are also possible long-term side effects of an impaired host immune system [4]. There is therefore a need for a greater understanding of the pathogenic mechanisms involved in RA so that new or improved therapies can be developed.

The characteristic pathology in RA is increased thickness of the synovial lining layer, which is normally two to three cells in depth and is composed of macrophages and FLSs (fibroblast-like synoviocytes). The sub-lining is infiltrated with numerous inflammatory cells including T- and B-cells, macrophages, endothelial cells and fibroblasts (Figure 1). This pathology is orchestrated by a complex interplay of inflammatory mediators including cytokines and chemokines, which establish an inflammatory loop within the joint. Specific cytokines can activate cells, including FLSs, to release proteases such as MMPs (matrix metalloproteinases) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) that degrade the extracellular matrix [5].

Twin studies have shown that RA has a significant genetic component, with a 12–15% disease concordance rate in MZ (monozygotic) twins compared with 4% in dizygotic twins [6,7]. The initial loci to be associated with RA risk were in the HLA (human leucocyte antigen) region, contributing at least one-third of the total genetic input [8], with the greatest risk coming from alleles of HLA-DRB1 [9]. Recent genome wide
Figure 1 | Pathogenesis of RA

A healthy joint (left-hand side) contains a small volume of synovium and a thin lining layer of FLSs. Within an RA-affected joint (right-hand side), rapid migration of immune cells into the sublining occurs. These attract more immune cells to the damaged area through the release of cytokines and chemokines, resulting in an inflammatory loop. Synovial hyperplasia is caused by increased numbers of macrophages and RA FLSs in the synovial lining layer. This leads to hypoxia (low levels of oxygen), another key feature of the synovium which, along with cytokine release, results in angiogenesis. Bone and cartilage loss is also a key feature of RA. This is due to the adhesion of RA FLSs followed by release of matrix-degrading proteases and inflammatory cytokines that cause osteoclast differentiation and cartilage and bone loss.

studies have identified a further 14 new susceptibility loci, containing genes involved in the immune and inflammatory responses [10], resulting in more than 40 RA susceptibility loci known to date.

Environmental influences on RA risk include smoking, which is associated with the development of anti-citrullinated positive disease. Conversely, alcohol consumption has been shown to reduce both the RA risk and its clinical severity [11].

RA fibroblast-like synoviocytes

Healthy synoviocytes provide the synovial joints with synovial fluid which contains plasma proteins and lubricating molecules such as hyaluronate [12]. Compared with fibroblasts from degenerative forms of arthritis, such as OA (osteoarthritis), RA FLSs have an auto-aggressive phenotype associated with transformed cells, which includes changes in morphology, proliferation rates and invasive potential [13]. Changes in gene expression also occur, including increased expression of anti-apoptotic proteins and proto-oncogenes, and reduced tumour suppressor gene expression [14]. The FLSs also secrete cytokines, most prominently IL-6 [15], adhesion molecules and matrix-degrading enzymes, e.g. MMP [16]. The release of cytokines and chemokines attracts circulating inflammatory cells to the inflamed joint, which can lead to further cellular activation and tissue damage.

The highly invasive nature of FLSs has been revealed using the SCID (severe combined immunodeficiency) mouse model, in which mice lack an adaptive immune system; RA FLSs were implanted with human cartilage into one flank of a mouse, with additional human cartilage implanted in the contralateral ‘control’ flank. After 60 days, the FLSs had invaded the control implant, indicating the long-term stability of their phenotype and that it is independent of adaptive immune cells. Moreover, invasion of control implant revealed the ‘metastatic’ potential of this cell type [17]. Further work revealed that this invasion is also independent of cell proliferation, determined by flow cytometry [18]. The stability and independence of the transformed phenotype in RA FLSs suggests that this change may be due to epigenetic modifications.

Epigenetic modifications in RA

Epigenetic modifications include DNA methylation and post-translational histone alterations, both of which control cell-specific gene expression patterns. The resultant cellular phenotype depends on a complex combination of different epigenetic modifications, termed the epigenetic signature.

Studies in MZ twins have revealed that changes in the epigenetic signature of peripheral blood leucocytes accumulate over time, particularly if the twins had lived apart early in life. This shows the effect of environmental factors and the idea of ‘epigenetic drift’ with age [19].

DNA methylation

DNA can be methylated at the 5-carbon of cytosine bases in eukaryotes via a methyl transfer reaction, resulting in the production of 5-methylcytosine. This reaction occurs at a cytosine base that precedes a guanosine, a CpG dinucleotide.

In normal cells, CpG dinucleotides are typically hypermethylated and are associated with repression of transcription [21]. CpG dinucleotides can be grouped into CpG islands,
Histone acetylation enzymes

The action of HATs allows the histone tails (colours) to disassociate from the DNA (black) and therefore allow access by TFs. The result is an acetylated nucleosome and transcriptional activation. HDACs remove acetyl groups (purple) and so cause the interaction of the histone tails with the DNA, resulting in no access for TFs. This causes deacetylated nucleosomes and transcriptional repression.

approximately 1 kb long, which are unmethylated and are common features of many gene promoters [22]. In almost all cancers, CpG dinucleotides have been shown to have reduced methylation levels, whereas CpG islands show increased levels [23]. The result is an aberrant transcriptional profile, which includes tumour suppressor genes becoming repressed and the genome becoming unstable [24]. Changes in DNA methylation may play a significant role in inflammatory diseases including asthma [25], and SLE (systemic lupus erythematosus) in which global DNA methylation levels are lower in patients [26].

The enzymes responsible for DNA methylation are DNMTs (DNA methyltransferases). There are three eukaryotic enzymes: DNMT1, DNMT3a and DNMT3b. DNMT1 functions as a maintainer of methylation [27], whereas DNMT3a and DNMT3b carry out de novo methylation [28]. However, these roles are not exclusive and it appears that the enzymes often work co-operatively [29,30]. DNA methylation was thought of as a highly stable epigenetic mark [31], but, since the discovery of a demethylation pathway, DNA methylation can now be considered as more of a dynamic change. The demethylation pathway is controlled by several enzymes, mainly methylcytosine dioxygenase TET (ten-eleven translocation) proteins, APOBEC (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like) [32] and DNA glycosylase MBD4 [33].

DNA methylation in RA

It has been reported that RA FLSs are globally hypomethylated compared with healthy FLSs and a microarray revealed that this change was associated with the up-regulation of 186 genes, including growth factors and matrix-degrading enzymes [34]. A DNA methylome study in RA FLSs revealed 1859 differentially methylated CpG motifs in RA compared with OA controls, with both hyper- and hypo-methylated genes present, including hypomethylation of genes encoding proteins involved in adhesion and migration [35], processes that are linked to the auto-aggressive FLS phenotype.

The expression of many retrotransposons, including LINE1 (long interspersed element 1), are regulated by DNA methylation. In RA FLSs, it has been found that there is a 30–300-fold increase in expression of LINE1 compared with healthy FLSs [36]. After inhibition of methylation at increasing concentrations, expression of LINE1 in RA FLSs increased in a dose-dependent manner. Further experiments have suggested that LINE1 may contribute to the aggressive phenotype of RA FLSs by regulating the SAPK (stress-activated protein kinase) [p38 MAPK (mitogen-activated protein kinase)] signalling cascades through currently unknown mediators; in FLSs, SAPK is part of a kinase pathway that enhances IL-6 and IL-8 [37], and MMP production [38].

Histone acetylation

Histones proteins undergo a variety of post-translational modifications by the action of ‘writer’ and ‘eraser’ proteins. The combination of these modifications is known as the ‘histone code’ and is read and interpreted by reader proteins to bring about DNA regulation.

One modification that has been of interest in several diseases to date is histone deacetylation. Histone tails can be acetylated and deacetylated by the action of two families of enzymes: the writers, HATs (histone acetyltransferases) and the erasers, HDACs (histone deacetylases) (Figure 2).
HATs catalyse the addition of acetyl groups from a donor acetyl-CoA on to lysine residues on histone tails. Acetyl groups are negatively charged, so when transferred onto the positive lysine, they neutralize the positive charge, resulting in decreased affinity for the negative phosphates of the DNA, freeing the DNA into an open conformation, allowing access by TFs (transcription factors) with resulting increased gene expression [39]. There are three major classes of HDACs that are grouped on the basis of their sequence similarity; Gcn5/PCAF [p300/CREB (cAMP-response-element-binding protein)–binding protein–associated factor], p300/CBP (CREB-binding protein) and MYST [40].

HDACs have the opposite activity, transferring the acetyl groups from histone tails back on to CoA. The removal of the acetyl group results in the positive histones interacting with the negative phosphate groups of DNA with resultant condensation of DNA and transcriptional repression [41]. There are four classes of HDACs, grouped on the basis of homology and cellular localization: class I (HDACs 1–3 and 8), which are localized to the nucleus, class II (HDACs 4–7, 9 and 10), which are present in both the nucleus and the cytoplasm, class III (SIRTs 1–7), which are structurally dissimilar, and, finally, class IV (HDAC11), which display some similarity to both class I and class II [42,43]. Most class I HDACs are targeted to histones when in a complex with TFs and other enzymes, e.g. HDAC1 is part of several complexes including Sin3a and NuRD (nucleosome remodelling and deacetylation) co-repressor complexes which are involved in transcriptional regulation and chromatin remodelling [44]. Many non-histone targets for HDACs have been identified [45], including the tumour suppressor gene p53; a gene involved in initiating apoptosis or cell-cycle arrest and which is down-regulated by HDAC-mediated deacetylation [46].

It is becoming increasingly evident that abnormal expression of HDACs has a role in regulating the inflammatory response. A significant reduction in HDAC activity has been found in peripheral lung tissue from patients with chronic obstructive pulmonary disease compared with non-smoking controls, but also between early and late stages of the disease [47]. Significant reductions in HDAC activity have also been noted in alveolar macrophages [48] and bronchial biopsies [49] from asthma patients compared with healthy controls. Inhibitors of HDACs have been shown further to attenuate inflammation in a murine model of allergic asthma [50]. Inhibition of HDACs in transformed cancer cell lines results in NF-κB (nuclear factor κ-B)–driven transcription of inflammatory genes [51]. It is clear that HDACs play a role in regulating inflammation and represent major new therapeutic targets.

**Histone acetylation in RA in vitro**

Initial studies on the role HDACs in RA has yielded contradictory results. RA synovial tissue in comparison with OA or healthy control tissue has shown a shift towards hyperacetylation owing to the decreased activity of HDACs and decrease in HDAC protein expression, particularly HDAC1 and HDAC2 [52]. Evidence from that study suggests that inhibiting HDACs would not be of benefit to patients. Conversely, another investigation in synovial tissue found the opposite; HDAC activity increased in comparison with OA and healthy controls [53]. The study also found that incubation with TNF further increased the activity of HDACs in RA FLSs. Those findings may explain the difference between the two studies as the former included patients who had been on anti-TNF therapy, which could account for their reduced HDAC levels. Furthermore, end-stage disease patients were used compared with active disease patients used in the latter study. End-stage RA tissue has been shown to have reduced cytokine production, e.g. IL–6, and reduced cell infiltration [54], which could potentially be influencing HDAC expression.

Studies in RA FLSs specifically demonstrate that expression of HDACs were not significantly different in RA FLSs compared with OA FLSs, with the exception of higher HDAC1 and lower HDAC4 [53,55]. Further studies knocking down HDAC1 and HDAC2 in RA FLSs using siRNA (small interfering RNA) transfection concluded that both HDACs play a role in enhancing cell proliferation and reducing apoptosis [55]. This suggests that therapeutics inhibiting HDACs could be important. In contrast with HDACs, expression of HATs in nuclear extracts did not vary in synovial tissue from RA, OA and healthy controls [53].

**Histone acetylation in RA murine models and human arthritic disease**

HDACis (HDAC inhibitors) have been used to treat neoplastic conditions, with SAHA (suberoylanilide hydroxamic acid; vorinostat) being the first FDA (Food and Drug Administration)-approved HDACi. It is currently used as a treatment for cutaneous T-cell lymphoma [56]. The actions of HDACis include increased apoptosis by up-regulation of pro-apoptotic genes (e.g. FasL) and reduced proliferation through inhibition of cell-cycle progression [57]. The use of such inhibitors is currently being considered for RA.

The class-I-specific HDACi (MS-275) showed promising results in the collagen-induced mouse model of RA, delaying onset of the disease and modulation of disease severity including inhibition of bone resorption, reduced joint swelling and lowered levels of pro-inflammatory cytokines [58]. Further work revealed that MS-275, when administered together with SAHA, inhibited proliferation of an RA FLS cell line in vitro [59].

ITF2357 (givinostat) is another HDACi that has been shown to be a very effective treatment in several RA mouse models. It results in reduced cartilage destruction, bone breakdown, joint swelling and pro-inflammatory cytokine expression by synovial cells and tissue. Furthermore, it reduced the total number of cells arriving at the inflamed joint [60]. More recently, a clinical trial of this agent in systemic-onset idiopathic juvenile arthritis resulted in reduced numbers of leucocytes, fewer active arthritic joints and reduced...
expression of inflammatory cytokines, while remaining safe and beneficial for the patient [61].

Currently, HDACis target certain classes of HDAC, but are not specific for individual enzymes. Understanding which HDACs contribute to RA will allow distinct targets to be identified so that more specific inhibitors can be developed.

**Conclusions**

Studies of the expression of HDACs in RA FLSs have produced opposing results; however, inhibiting HDACs using HDACis in animal models suggests that HDACs do play an important role in the RA phenotype. The evidence described above shows that this is an area that justifies further evaluation as HDACs are a potential important new therapeutic target in this chronic inflammatory condition. A surge in investigations is therefore required to determine the effects of individual HDACs in RA FLSs.

**References**


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