

Aberrant epigenetics in rheumatoid arthritis and osteoarthritis

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Evaluation of: Huber LC, Brock M, Hemmatazad H et al.: Histone deacetylase / acetylase activity in total synovial tissue derived from rheumatoid arthritis and osteoarthritis patients. *Arthritis Rheum.* 56(4), 1087–1093 (2007).

Reduced activity of histone deacetylase (HDAC) was observed in both rheumatoid arthritis (RA) and osteoarthritis (OA). This was the first study that disclosed aberrant epigenetics in RA and OA. However, with limited sample size, such interesting findings need to be confirmed in a larger cohort of patients. HDAC inhibitors appear to be a therapeutic strategy for RA although they may not alter the disease progression of OA. Therefore, reduced HDAC activity might not contribute to the pathogenesis of RA and OA. As HDAC inhibitors were originally designed for oncological indications, they might not be well tolerated in long-term RA therapy. Thus, it is important to discover the molecular therapeutic mechanisms of HDAC inhibitors in RA. This is in the hope that, with a better understanding of the antirheumatic mechanisms, new generations of HDAC inhibitors with better specificities and safety profiles could be developed for the management of RA.

Aberrant epigenetics have recently been reported in rheumatoid arthritis (RA) and osteoarthritis (OA) [1]. The term epigenetics is used to describe all meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself [2,3]. Three systems, namely DNA methylation, RNA-associated silencing and histone modification, are involved in epigenetic regulation [2,3]. In eukaryotic cells, the genomic DNA is wrapped around a histone octamer to form nucleosomes, which make up the basic structural unit of chromatin [3–5]. The amino ends of the nucleosomes are vulnerable to a variety of post-translational modifications [3–5]. These include acetylation by histone acetyltransferase (HAT), deacetylation by histone deacetylase (HDAC) and methylation by histone lysine methyltransferase, as well as phosphorylation, polyadenosine diphosphate ribosylation, ubiquitinylation, sumoylation, carbonylation and glycosylation [3–5]. Histone acetylation status is one of the determinants that controls gene expression [3–5]. Acetylation of the ϵ -NH₂ group on lysine residues within histone tails neutralizes the positive charge, loosens chromatin and facilitates the access of various transcriptional factors to the promoter regions of their respective target genes [3–5]. Conversely, deacetylating the lysine residues on histone tails leads to chromatin condensation and transcription repression [3–5].

Past investigations of human disease mainly focused on genetic mechanisms [2]. However, abnormalities in epigenetic regulation could also contribute to the development of various

diseases [2–4]. Aberrant recruitment of HDACs to promoters has been reported in the pathogenesis of acute promyelocytic leukemia, and acute myeloid leukemia, as well as B-cell lymphoma [5]. Moreover, overexpression of HDAC has also been reported in prostate, gastric, breast, colorectal and cervical tumor [5]. Therefore, downmodulation of HDAC activities appears to be a practical strategy for cancer management. This subsequently led to the development of HDAC inhibitors as anti-cancer drugs [3–5]. Besides vorinostat (suberoylanilide hydroxamic acid [SAHA], Merck, NJ, USA), which had been approved by the US FDA for the management of cutaneous T-cell lymphoma, approximately ten other HDAC inhibitors are currently undergoing Phase I–III clinical investigations for various oncological indications [3–8]. On the other hand, underexpression or decreased activities of HDACs might contribute to the pathogenesis of inflammatory lung diseases [9–11]. As aberrant histone acetylation/deacetylation was found to be implicated in various diseases, it will be interesting to find out the impact of HDAC and HAT in RA and OA.

Huber and coworkers carried out an ice-breaking study to assess the activities of HAT and HDAC in synovial tissues derived from RA and OA patients [1]. They found that HDAC activity was suppressed in both RA and OA, while HAT activity remained unchanged [1]. The balance of HAT over HDAC activity was strongly skewed towards histone hyperacetylation in patients with RA [1]. The authors claimed such results offered innovative molecular insights into

the pathogenesis of RA, which in turn might be relevant to the development of future therapeutic approaches [1]. Based on this study, one could easily deduce that upmodulation of HDAC or downregulation of HAT might offer a therapeutic strategy for RA and OA, as downregulation of HDAC was observed with these diseases. However, anti-inflammatory effects through HDAC inhibition had been documented in several independent studies utilizing various pre-clinical RA models. The perspectives on HDAC inhibition, together with its effects on RA/OA pathogenesis, as well as the use of HDAC inhibitors as a therapeutic approach for RA, will be discussed in this article.

Results from the paper

The activities of HDAC and HAT were measured in nuclear extracts of total synovial tissue samples derived from RA ($n = 7$), OA ($n = 6$) and arthritis-free control patients ($n = 5$). The expression of HDAC-1 and -2 was quantified by Western blotting and immunohistochemistry was performed for HDAC-2. The HDAC activities were 1.5 ± 0.3 , 3.2 ± 0.7 and 7.1 ± 4.2 $\mu\text{mol}/\mu\text{g}$ in RA, OA and arthritis-free synovial tissues, respectively. The differences in HDAC activities between RA and control and OA and control were statistically significant (RA vs control $p = 0.01$; OA vs control $p = 0.008$). However, HAT activity remained unchanged in RA, OA and controls. The ratio of HDAC activity over HAT activity dropped significantly by $12 \pm 2\%$ and $26 \pm 3\%$ in RA and OA, respectively, in comparison with arthritis-free controls (RA vs control $p = 0.002$; OA vs control $p = 0.009$). Furthermore, the expression of HDAC-1 and -2 was clearly lower in RA samples than in OA samples.

Significance of the results

This is an important study as it provides new clues for better understanding of the molecular mechanisms underlying RA and OA. Aberrant HDAC or HAT activity/expression has been observed in various diseases [2–5,9–11], and they may contribute to disease development and progression. Before the aforementioned study, nothing was known about the influence of HDAC or HAT on the pathogenesis of RA and OA. Huber and colleagues reported that HDAC activity was suppressed in both RA and OA, implying that downregulation of HDAC might be involved in the pathogenesis of the diseases. If this is true, upmodulation of HDAC or downregulation of HAT appears to be a therapeutic approach in both RA and OA.

However, it is too early to conclude that RA or OA is indeed associated with reduced HDAC activity in synovial tissues. The study carried out by Huber and coworkers only had a very small sample size (RA: $n = 7$; OA: $n = 6$ and arthritis-free control: $n = 5$). Such limited numbers of patients may not represent the whole population. Besides, the small sample size does not provide good statistical efficiency. It will also increase the occurrences of random errors and, hence, obtaining a significant result owing to chance spuriously increases. The subjects were obtained within the same hospital, which could have already generated some form of selection biases. In addition, there was an imbalanced gender distribution among the patients. The majority of RA and OA patients were females (RA: six of seven; OA: five of six; control: two of five). Furthermore, the synovial tissues were not derived from the same type of joints. In addition, all the aforementioned subject differences were not adjusted or controlled for in the statistical analyses. To date, we do not know whether gender and the location of the joints affects HDAC or HAT activity and expression. Therefore, the interesting findings obtained by Huber and coworkers need to be confirmed in a larger cohort of patients.

Future perspective

The relationship between the pathogenesis of RA/OA and reduced HDAC activity in synovial tissues remains unknown. Its current situation appears to be a 'the chicken or the egg' debate [1]. Decreased HDAC activity could contribute to disease development and joint destruction. However, these could also be epiphenomenon of ongoing inflammation in the joints. Although the precise etiologies of both RA and OA remain largely unknown, the pathogenesis of RA is very different from that of OA. OA is a degenerative disease that leads to cartilage destruction, while RA is an autoimmune disease characterized by synovial hyperplasia, influx of inflammatory leukocytes and pronounced angiogenesis. The proinflammatory cytokines tumor necrosis factor (TNF)- α and interleukin (IL)-1 β are crucial to its pathogenesis. Therefore, it is hard to establish that decreased HDAC activity could mediate the pathogenesis of two such apparently distinct diseases.

If downregulation of HDAC really contributes to the pathogenesis of RA/OA, further inhibition of HDAC would potentiate RA/OA-disease progression. However, a myriad of experiments carried out by independent investigators did not

support such a conjecture. The HDAC inhibitors phenylbutyrate and trichostatin A, as well as FK-228 (Gloucester Pharmaceuticals, MA, USA) could inhibit the *in vitro* proliferation of synovial fibroblasts derived from RA patients or rats with adjuvant-induced arthritis (AIA) at noncytotoxic concentrations [12,13].

The *in vivo* antirheumatic activities of HDAC inhibitors were also documented. Topical treatment of phenylbutyrate (200mg/paw, twice daily $\times$ 18 days) and trichostatin A (100mg/paw, twice daily $\times$ 18 days) administered either prophylactically or therapeutically could reduce joint swelling, decrease subintimal mononuclear cell infiltration, inhibit synovial hyperplasia, suppress pannus formation and prevent cartilage or bone destruction in rats with AIA [13]. Similarly, FR235222 (Astellas Pharma Inc., Tokyo, Japan), a fungal metabolite with HDAC-inhibitor activity, alleviated joint swelling induced by AIA in rats [14]. In mice with antibody-mediated arthritis, therapeutic intervention with a single intravenous injection of FK-228 (2.5 mg/kg) suppressed joint swelling, synovial inflammation, bone damage and cartilage destruction [12]. Subsequently, we tested SAHA and MS-275 (Bayer Schering Pharma, Berlin, Germany) in collagen-induced arthritis models [15]. SAHA only had moderate prophylactic activities in both mice and rats [15]. MS-275 displayed potent antirheumatic activities [15]. In prophylactic studies, high doses of MS-275 (rat: 3mg/kg; mice: 10mg/kg) prevented bone erosion and markedly delayed the onset of arthritis, while low doses of MS-275 (rat: 1mg/kg; mice: 3mg/kg) strongly attenuated joint swelling, bone erosion and bone resorption that was accelerated by arthritis [15]. In histological analyses, we

did not find synovial hyperplasia, panus formation, cartilage or bone destruction in the mice treated with MS-275 (10mg/kg) as prophylaxis [15]. In therapeutic intervention, MS-275 (5 mg/kg) stopped the disease progression and joint destruction in rats, displaying even greater disease-modifying effects than methotrexate [15]. In summary, HDAC inhibitors appear to be an innovative therapeutic strategy for RA. Although the molecular antirheumatic mechanisms of HDAC inhibitors were not extensively studied, upregulation of cell-cycle inhibitors p16 and/or p21 in transformed synovial fibroblasts and suppression of proinflammatory cytokines TNF- α , IL-1 β and -6 by HDAC inhibitors could contribute to their antirheumatic activities [12,13,15]. In addition to RA models, we also assessed SAHA and MS-275 in a rat meniscectomy-induced OA model [Unpublished Data]. Neither SAHA nor MS-275 had destructive or protective effects on cartilage damage induced by meniscectomy. Therefore, reduced HDAC activities in RA and OA synovial tissues might not contribute to the pathogenesis of RA and OA.

Currently, HDAC inhibitors are attracting significant interest as anti-inflammatory agents. Besides RA models, the anti-inflammatory activity of HDAC inhibitors has also been studied in preclinical models for inflammatory bowel diseases, systemic lupus erythematosus, hepatitis, stroke and multiple sclerosis [16–18]. However, HDAC inhibitors might also exhibit proinflammatory activities in microglia and airway inflammation [16–18]. Therefore, the targeted diseases and tissue types determine whether HDAC inhibitors work as a pro- or anti-inflammatory agents.

Executive summary

This was the first study that disclosed aberrant epigenetics in rheumatoid arthritis & osteoarthritis

- Reduced histone deacetylase (HDAC) activity was observed in both rheumatoid arthritis (RA) and osteoarthritis (OA).
- Unchanged histone acetyltransferase activity was found in both RA and OA.

Impact of histone deacetylase inhibitors in rheumatoid arthritis & osteoarthritis

- Various HDAC inhibitors have antirheumatic activity in RA models.
- HDAC inhibitors suberoylanilide hydroxamic acid and MS-275 did not potentiate or suppress cartilage damage in an OA model.

Conclusion

- Reduced HDAC activities in both RA and OA might not contribute to the pathogenesis of RA and OA.
- HDAC inhibitors appeared to be a therapeutic strategy for RA.

Future perspective

- Discovering the molecular antirheumatic mechanisms of HDAC inhibitors in RA, is the next challenge facing researchers.
- New generations of HDAC inhibitors with better specificities and safety based on a better understanding of antirheumatic mechanisms must be developed.

The clinical effects of HDAC inhibitors have never been determined in RA. Since originally designed for oncological indications, the adverse effects of HDAC inhibitors in humans cannot be ignored. Given that RA is an incurable disease requiring life-long therapy, the toxicities of HDAC inhibitors may not be well-tolerated

long-term. Therefore, it is important to determine the molecular therapeutic mechanisms of HDAC inhibitors in RA. This is in the hope that, with a better understanding of the antirheumatic mechanisms, new generations of HDAC inhibitors with better specificities and safety profiles could be developed for the management of RA.

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