13. Targeting the Epigenetic Modifications of Synovial Cells

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SUMMARY

Rheumatoid arthritis (RA) is a systemic inflammatory disease that mainly affects the synovial tissues of joints. Like in other autoimmune-related disorders, both the etiology as well as the pathogenesis of RA has not yet been completely unravelled. It is generally accepted, though, that autoimmune disorders develop through a combination of the individual genetic susceptibility, environmental factors, and dysregulated immune responses.

Genetic predisposition has been described in RA, in particular as "shared epitope", a distinct sequence of amino acids within the antigen presenting peptide groove of the major histocompatibility complex (MHC). Imbalanced immunity is reflected by the production of autoantibodies and the accumulation of reactive helper T cells within the rheumatoid synovium. In addition, environmental factors have been postulated as disease modulating agents, including smoking, nutrition and infectious agents. So far, these factors have been studied almost exclusively as separate agents.

However, the way genes are transcribed can be affected by environment, nutrition, and ageing – without changes in the nucleotide sequence of the underlying DNA. These patterns of alterations in the gene expression profiles are called *epigenetics*. The term *epigenetics* is used to refer to molecular processes that regulate gene expression patterns, however without changing the DNA nucleotide sequence. These epigenetic changes comprise the postsynthetical methylation of DNA and posttranscriptional modifications of histones, including methylation, phosphorylation, ubiquitination, sumoylation, biotinlyation and, most importantly, *deacetylation and acetylation*. With respect to the complex pathogenesis of rheumatic diseases, the *epigenome* is an emerging concept that integrates different etiologies and, thus, offers the opportunity for novel therapeutic strategies. Based on the fact that current therapies have not resulted in an ACR 70 above 60% and have never been targeting the

activated synovial fibroblast, novel therapeutic strategies should target the epigenetic pathways of synovial activation in RA.

KEYWORDS

epigenome, rheumatoid arthritis (RA), synovial fibroblasts (SF), histone modifications, DNA methylation, novel therapeutic strategies

1. INTRODUCTION

Less than a decade ago, the number of genes that is encoded within the nucleus of a single human cell was estimated to at least 100'000 genes or more. Much hope for our understanding of pathogenesis and treatment of diseases thus was put on the successful accomplishment of the human genome project. It was therefore most surprising that the number of genes finally detected was quite low. Most of the 25000 genes identified encode biological functions that remain undiscovered so far and the functional characterization of these genes in normal physiology as well as in the pathogenesis of diseases remains the main issue for biomedical research in the next years [1]. However, this approach through "*functional genomics*" might be biased by postreplicational, posttranscriptional as well as posttranslational modifications – and proteome diversity due to alternative splicing of mRNA transcripts and other biochemical alterations somehow limits the utility of genomic information [2, 3]. The question how the genome integrates intrinsic and environmental factors thus might be answered by the emerging concept of the *epigenotype*. The term *epigenetics* comprises stable alterations of the genetic information that are heritable but do not involve mutations of the DNA sequence itself. Epigenetic regulations are mediated by several biochemical phenomenona, most importantly however by *histone modifications* and *DNA methylation*. Epimutations and epigenetics are required for development and differentiation of cells within a multicellular organism. Moreover, they allow a cell to respond to environmental

stimuli throughout adult life by means of stable expression or repression of genes in specific cell types. Currently, no epigenetic information is systematically analysed and epigenetic modifications have not been assessed within the human genome project. Since epigenetics might play the linking role between environmental factors and genetics in determining a certain phenotype, the investigation of epigenetic alterations along the lines of chromosome-wide and promoter specific arrays will represent an fascinating area of future research. Already, pilot studies for the human epigenome project have been undertaken [4]. In this regard, the epigenome could provide a readout of an individual's environmental history [5] and the convential method of studying human diseases by molecular genetic approaches and additional environmental factors could soon be extended to a novel field of *epigenetic epidemiology*.

Within this chapter, we focus on the emerging concept of epigenetics and its implications for potential treatment strategies in rheumatoid arthritis (RA).

2. DEFINITIONS

The term *epigenetics* is used to refer to molecular processes that regulate gene expression patterns, however without changing the DNA nucleotide sequence. The epigenome of a cell is defined by two major groups of biochemical alterations: postsynthetic methylation of DNA and modifications of histones that package DNA and, thus, modulate the accessibility for transcription factors to information present on nucleid acid . These modifications are mitotically heritable and can be transmitted during cell division from one generation of cells to the next. Both physiological and pathological responses to environmental stimuli are probably mediated by epigenetic mechanisms. Even in the absence of such environmental factors, the epigenomic profile is reversible and highly variable, probably due to stochastic events in the somatic inheritance of epigenetic profiles [6]. The remarkable degree of variation distinguishes *epimutations* and *epigenetic changes* from "true" genetic mutations.

From a therapeutic view, finally, the reversibility of epigenetic changes offers the opportunity of using pharmacological strategies to revolve a certain phenotype [7]. The dynamic interaction between all factors involved is illustrated in Figure 1.

Insert Figure 1

2.1. DNA METHYLATION [8]

DNA methylation in eukaryotes is a post-replication modification restricted to the pyrimidine base cytosine within the dinucelotide sequence CpG, , which form clusters of genetic regions (CpG islands) that surround the promoter region of protein-coding genes.

Phenotypic analyses of several DNA methyltransferase (DNMT) enzymes have revealed interesting mechanistic insights into epigenetic methylation: DNMT3a and 3b for example are de novo methyltransferases and introduce cytosine methylation at CpG sites that were previously unmethylated. DNMT1 acts during replication and cell division by copying existing methyltransferase pattern onto newly synthesized DNA strands. For the process of DNA methylation, DNMTs use S-adenosylmethionine as a methyl donor, whose generation is mainly modulated by the availability of different methyl donors. Briefly, methyltetrahydrofolate governs the conversion of homocysteine to methionine, which is further metabolized to S-adenosylmethionine. Deficiencies in the enzymes involved in these processes result in hypomethylation of DNA. Several nutrients play key roles within this metabolism. The major dietary sources of methyl groups include folate, choline and vitamin B12 [9-12].

The insertion of a methyl group at position five of the cytosine ring leads to structural changes of chromatin and is associated with gene repression. This silencing function on the level of

gene expression can be achieved by different mechanisms. Structural modifications of the DNA might block the proper docking of DNA-binding factors to their fitting recognition sites, thus inhibiting gene transcription, whereas Methyl-CpG-binding proteins (MBPs, such as MeCP2, MBD1-4) function redundantly as transcriptional co-repressors. Moreover, MBPs have been shown to interact with enzymes regulating histone modifications. This interaction could provide a link between different epigenetic mechanisms.

2.2. HISTONE MODIFICATIONS [13, 14]

Apart from DNA methylation, local chromatin architecture and, thus, transcriptional regulation of gene expression is strongly influenced by covalent biochemical modifications subsumed under the term *histone code*. These epigenetic changes include posttranscriptional modifications of histones, including methylation, phosphorylation, ubiquitination, sumoylation, biotinlyation and, most importantly, *deacetylation and acetylation* [14, 15]. *Nucleosomes* are the fundamental building blocks of the heterochromatin consisting of an octamer of four core histones and DNA. This octameric structure is made out of a H3-H4 tetramer and two H2A-H2B dimers. Histone H1 has a linker function between DNA and protein and governs the path of the DNA as it exits from the nucleosome. With respect to histone modification, the DNA of physiologically resting cells is wrapped tightly around the core histones, thus preventing the binding of basal transcription factors (e.g. the TATA box binding protein) and RNA polymerase II [16]. Gene transcription is initiated when histones are modified in order to create an open, accessible form of chromatin. Probably the most investigated modification of the histone code is the (de)acetylation of core histones. Histone acetylation is performed by histone acetyltransferases (short: histone acetylases, HATs) that neutralize positive charges at the ε amino groups of lysine residues at the N-termini. Hyperacetylation of histones is generally associated with enhanced rates of gene

transcription. Conversely, the space between histones and surrounding DNA is reduced by de- and hypoacetylation – and transcription factors are sterically hindered of binding, leading to gene silencing. Taken together, the gene transcription rate is regulated by the balance between histone acetylation and histone deacetylation. The targeted deacetylation of histones is performed by several multisubunit enzyme complexes, i.e. histone deacetylases (HDACs) [15, 17]. Figures 2 shows the dynamic interplay of epigenetic mechanisms between states of silent and transcriptionally active chromatin.

Insert Figure 2

Eukaryotic members of the HDAC family can be divided into three major groups, of which class I and class II HDACs so far comprise the best characterized classes with respect to function. The class I comprises HDAC1, 2, 3, and HDAC 8 and localize almost exclusively within the nucleus to exert their function. HDACs 4, 5, 6, 7, 9, and 10 are subsumed under the term class II HDACs, which are mainly found in the cytoplasm and are shuttled into the nucleus when needed. Whereas class I HDACs are expressed in most cell types, the expression pattern of class II HDACs appears to be tissue-specific, indicating a possible role in cellular differentiation and development. HDACs remove the acetyl group from the nucleosomal core histones by using a sophisticated charge-relay system using Zn^{2+}-ions as prosthetic group. HDAC inhibitors such as Trichostatin A (TSA) fit into the active catalytic pocket of the enzyme, exchange the zinc ion and, thus, make the system dysfunctional [18].

HATs, on the other hand, comprise a family of proteins that catalyze the acetylation of lysine residues of one of the core histone proteins [19]. Traditionally, HATs are categorized in two groups based on there subcellular localization: type A, located in the nucleus and type B, located in the cytoplasm. Nuclear type A-HATs acetylate nucleosomal histones within

chromatin in the nucleus and thus type A HATs are related to transcriptional regulation processes. On the other hand, type B HATs acetylate newly synthesized free histones in the cytoplasm. Since recent data indicate that HAT activity can be induced also in multiple protein complexes that are related to transcriptional processes, this historically categorization is no longer used. Amino acid sequence analyses of all HAT proteins revealed the important feature that HAT proteins fall into distinct families that share relatively poor sequence similarity. All three superfamilies (GNAT, MYST and p300/CBP-HATs) however have a highly conserved acetyl-CoA binding site in common.

The best-understood family of HATs is the GNAT (general control nonderepressilble -5 (Gcn5)-related N-acetyltransferase) superfamily. Humans express two (Gcn5)-like acetyltransferases: Gcn5 and PCAF (p300/CBP associated factor). Both of these proteins can interact with an other HAT protein complex, i.e. p300/CBP. P300/CBP is a ubiquitously expressed, global transcriptional co-activator that regulates cell cycle, differentiation and apoptosis. The HAT activities of p300/CBP enables the transactivation of DNA binding transcription factors (p53, E2F, myb, GATA1, Rb) as well as the acetylation of all 4 histone proteins. Mutations in the HAT active site inhibit their transcriptional activating function.

The MYST family of HATs is particularly interesting as these proteins show similarity with other acetyltransferases exclusively within the acetyl-coenzyme A binding motif. The members of the MYST family named this HAT group: MOZ (monocytic leukemia zinc finger protein), Ybf2/sas3, Sas2, and Tip60. MOZ is involved in the chromosome translocations associated with acute myeloid leukemia. MOZ acts as a transcriptional coactivator for AML1, which is essential for establishment of definitive hematopoiesis. An overview of the different HAT families is provided in Table 1.

Insert Table 1

Finally, DNA methylation and histone modifications have been considered as two distinct mechanisms, which influence the level of gene expression in an independent manner. It was shown however that HDACs are correlated to DNA methylation, either through direct interaction of HDACS with DNMTs or by the function of Methyl-CpG-binding proteins (MBPs). Another line of evidence suggesting that MBPs interact with HDAC1 and HDAC2 to recruit the Sin3 corepressor protein further supports a link between DNA methylation and histone modifications [20, 21]. Thus, DNMTs appear to be some kind of dual function proteins, which are recruited by transcriptional repressors. On the other hand, they have a non-enzymatic function interacting with histone methyltransferases and HDACs, hence leading to chromatin remodelling [22, 23].

3. EPIGENETIC MODIFICATIONS in RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a systemic disease mainly affecting the synovial tissues of joints. The process of ongoing inflammation and erosion of articular cartilage and subchondral bone causes severe pain, functional impairment and ultimately disability [24]. Like in other autoimmune-related disorders, both the etiology as well as the pathogenesis of RA has not yet been completely unravelled. It is generally accepted, though, that autoimmune disorders develop through a combination of the individual genetic susceptibility, environmental factors, and dysregulated immune responses [25, 26].

Genetic predisposition has been described in RA, in particular as "shared epitope", a distinct sequence of amino acids within the antigen presenting peptide groove of the major histocompatibility complex (MHC). Imbalanced immunity is reflected by the production of autoantibodies such as rheumatoid factors and cyclic citrullinated peptides as well as by the accumulation of reactive helper T cells within the rheumatoid synovium. In addition, environmental factors have been postulated as disease modulating agents, including smoking, nutrition and infectious agents. So far, these factors have been studied almost exclusively as

separate disease driving agents. As mentioned before, however, the way genes are transcribed can be affected by environment, nutrition, and ageing that influence the epigenetic patterns.

In this regard, Kim and coworkers investigated whether methotrexate induces genomic DNA hypomethylation in patients with inflammatory arthritis [27]. Methotrexate is an immunosuppressive agent, which is used in the treatment of RA in addition to glucocorticosteroids and biologicals. Methotrexate exerts its function by blocking the enzyme dihydrofolat reductase thus interrupting the methyl transfer function of folate. Within the study in question, the amount of methylated genomic DNA was lowest in subjects with inflammatory arthritis who were not taking methotrexate, highest in subjects with inflammatory arthritis who were taking methotrexate, and intermediate in control subjects. Surprisingly, these data indicate that inflammatory arthritis might be associated with genomic DNA hypomethylation, which can be reversed with methotrexate [27]. Since methotrexate would be expected to inhibit transmethylation, the results from this study are somewhat paradoxical.

In an animal model of adjuvant arthritis, treatment with the combination of tryptophan, methionine and methotrexate caused a drastically reduced course of arthritis, whereas treatment with methotrexate alone exerted only a slight inhibitory effect [28]. The methyl groups transferred during methylation of DNA are ultimately derived from methionine. Therefore, high methionine intake was suggested to increase DNA methylation. Because of the distinct biochemistry within the methionine cycle, methylation of DNA probably is impaired under dietary excess of methionine by inhibiting remethylation of homocysteine [29]. Mature B-lymphocytes and plasma cells express CD21 (complement receptor II) on the cell surface to exert their function as (auto-)antibody producing cells. CD21 binds the complement component C3d, which is expressed within immune complexes. Interestingly, immature precursor of B cells, such as pro-, pre-, or plasma B lymphocytes do not express CD21, probably because they contain a methylated CpG island within its promoter region. When

peripheral blood mononuclear cells and synovial fluid mononuclear cells from patients with RA were investigated, however, the CD21-CpG island was found to be demethylated [30]. These data suggest a role of these cells in the dysregulation of the immune response in RA patients.

It is of interest to note here that most therapies have been targeting T- and B-lymphocytes and/ or monocytes/ macrophages and their respective proinflammatory cytokines, but never the synovial fibroblast, which was shown to be activated even in the absence of stimulating immune cells and cytokines [31]. This observation has prompted us to study signaling pathways in RA-SF. Since DNA methylation is also involved in the regulation of endogenous retroviral sequences, which have been suggested to play a role in the induction of autoimmune diseases, we searched for the presence of such sequences. By screening RA synovial fluid pellets, a homologue to the human retrotransposable L1 element was found by our group [32]. Human L1s (or LINE-1, long interspersed nuclear elements) are poly(A)-retrotransposons lacking long terminal repeats. L1s contain an untranslated region (UTR) and two open reading frames (ORF1 and 2), the latter one encoding for a protein with endonuclease and reverse transcriptase activity. Inhibition of the DNA methyltransfer by 5-aza-2'-deoxycytidine induced the expression of L1s by DNA hypomethylation. It was further shown that three of five CpG islands of the genomic L1 5'-UTR were hypomethylated in RA synovial fibroblasts. Moreover, L1 retroelements induced the expression of p38δ, one of the four isoforms of mitogen-activated protein kinases as well as galectin-3 binding proteins [32, 33].

With respect to histone acetylation, Ito and coworkers could show that reduced activity of HDAC class I enzymes leads to enhanced transcription of genes encoding inflammatory proteins, such as tumor necrosis factor (TNF)-α, interleukin (IL)-8 and matrix metalloproteinase (MMP)-9 [34]. These studies were performed in lung tissue derived from patients with inflammatory and obstructive pneumopathies such as asthma bronchiale and chronic obstructive pulmonary disease (COPD). Our laboratory has investigated HDAC

activity levels in total synovial tissue of patients with RA [35]. When we compared these samples with the respective levels in patients with osteoarthritis and normal synovial tissue, we found a significant decrease of total HDAC activity. This was probably due to reduced expression of HDAC 1 and 2 proteins in synovial tissue. Conversely, several studies have proposed HDAC inhibitors as beneficial agents for the treatment of RA and other inflammatory processes [36, 37].

It remains unclear, whether the observed reduction in HDAC activity is "the chicken or the egg" in the pathogenesis of RA and it can not be excluded that the reduced HDAC activity levels reflect an epiphenomenon of ongoing inflammation [35]. Evidence for a key role of histone modifications is based on the fact that the pro-inflammatory transcription factor *nuclear factor kappa B* (NFκB) is highly activated in RA synovial cells (for review see [24]). Since Ito et al have further shown that HDAC 2 suppresses the NFκB-mediated gene expression [38], we speculate that class I HDACs appear to act upstream of NFκB and other related transcription factors in RA. Thus, HDAC activity appears to play a key role in the pathogenesis of autoimmune-related joint diseases.

Jungel and coworkers finally have shown that co-treatment of RA-SFs with the HDAC inhibitor TSA and TNF-related apoptosis inducing ligand (TRAIL) induced programmed cell death (apoptosis) in a synergistic manner. When used alone, TRAIL and TSA exhibited no or only a modest effect [39].

Regulatory T cells (Tregs) have been thought to be of potential benefit for the treatment of autoimmune diseases and Foxp3 has been proposed as a master regulator governing both development and function of CD4-positive T lymphocytes. In this regard, it was recently shown that the expression of Foxp3 has to be stabilized by complete demethylation of CpG islands within this promoter region in order to develop a permanent suppressor cell lineage. These findings might be of clinical importance with respect to the therapeutical transfer of T regs in autoimmune diseases [40].

Taken the data on epigenetic modifications on RA-SF together with the fact that all current therapies, including the novel biologics, do not result in an ACR 70 greater than 60%, and that the RA-SF has never been targeted by any therapeutic strategy, future efforts should be given to target the activated RA-SF [41].

4. PERSPECTIVES

The current method of investigating the pathogenesis of diseases includes molecular genetic analyses to identify disease-specific gene sequences on one side and epidemiological approaches to detect potential environmental factors on the other hand. These efforts have only been partly successful to find explanations for the complex pathogenesis of autoimmune-related diseases such as RA. Epigenetic modifications thus appear to play pathogenetic key roles in genetically predisposed individuals. Distinct from genetic mutations, which are permanent, epigenetic alterations show an *intrinsic plasticity* and might well be targeted by *pharmacological strategies*. Various drugs that modulate the epigenetic reactions in rheumatic arthritis have already been tested in vitro and in animal models. In particular, targeting DNA methylation and histone acetylation are clearly within future therapeutic prospects. Several epigenetic drugs have also been approved by the FDA, in particular for clinical trials addressing the treatment of malignancies (reviewed in [42]). Nucleoside analogues such as azacytidine as well as the first orally bioavailable inhibitor zebularine inhibit DNA methylation by being incorporated into replicating DNA. These agents have been tested successfully in the treatment of hematologic cancers and myelodysplastic syndromes. Another group of DNA methylation inhibitors includes non-nucleoside analogues that inhibit DNA methyltransferase enzyme activity in order to exert their function. These drugs are already in clinical use and comprise the anesthetic agent procaine, procainamide (an antiarrhythmic compound) and the antihypertensive drug hydralazine. It has been reported that these agents cause global DNA hypomethylation in cancer cells as well as T lymphocytes in different experimental systems. On the other hand, various HDAC inhibitors are currently in phase I/ II of clinical trials. These inhibitors include TSA, suberoylanilide hydroxamic acid (SAHA), valproic acid, phenylbutyrate and others. SAHA, for example, was accepted by the FDA only recently for the treatment of advanced cutaneous T-cell lymphoma. Other studies have

revealed strong anticancer activities of HDAC inhibitors and feasible pharmacokinetic properties for the treatment of hematologic tumors. However, it is important to stress that all inhibitors, which are currently used, block the different HDAC enzymes without any preference for specific isoforms. To provide a more specific approach for future therapies, novel compounds have to be developed.

In addition, the combination of agents that block both DNA methylation and histone modifications might be of therapeutical interest. TSA, which has long been considered as a specific histone deacetylase inhibitor leading to histone hyperacetylation and activation of unmethylated gene sequences, has been shown to induce systemic and replication-independent demethylation of DNA [43] . The results from this study should be taken into account when novel, TSA-related epigenetic drugs are designed.

Finally, dietary recommendations for putative epigenetic drugs as found in green tea, garlic, broccoli and other phytochemical compounds [44, 45] might be an additional option apart from pharmaceutical interventions.

Until then, however, novel biologicals that reverse the epigenetic pattern have to be designed. Moreover, hurdles facing in vivo efficacy and toxic side effects have to be overcome. Along the line of chromosome-wide and promoter specific arrays, the epigenotype has to be investigated, especially with respect to the activated RA-SF. This will open the scope for future therapies and might push epigenetic inhibitors as potent agents to treat or prevent disease on an individual basis.

5. REFERENCES

1. Huber, L.C.,Distler, O. (2006) Rheumatology related genes, Identification. In: D Ganten, K Ruckpaul (eds). *Encyclopedic Reference of genomics and proteomics in molecular medicine* Springer:1670-1677.

2. Southan, C. (2004) Has the yo-yo stopped? An assessment of human protein-coding gene number. *Proteomics* 4:1712-26.

3. Little, P.F. (2005) Structure and function of the human genome. *Genome Res* 15:1759-66.

4. Rakyan, V.K., Hildmann, T., Novik, K.L., Lewin, J., Tost, J., Cox, A.V., Andrews, T.D., Howe, K.L., Otto, T., Olek, A., Fischer, J., Gut, I.G., Berlin, K.,Beck, S. (2004) DNA methylation profiling of the human major histocompatibility complex: a pilot study for the human epigenome project. *PLoS Biol* 2:e405.

5. Whitelaw, N.C.,Whitelaw, E. (2006) How lifetimes shape epigenotype within and across generations. *Hum Mol Genet* 15 Spec No 2:R131-7.

6. Wong, A.H., Gottesman, II,Petronis, A. (2005) Phenotypic differences in genetically identical organisms: the epigenetic perspective. *Hum Mol Genet* 14 Spec No 1:R11-8.

7. Ballestar, E., Esteller, M.,Richardson, B.C. (2006) The epigenetic face of systemic lupus erythematosus. *J Immunol* 176:7143-7.

8. Klose, R.J.,Bird, A.P. (2006) Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci* 31:89-97.

9. Niculescu, M.D.,Zeisel, S.H. (2002) Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. *J Nutr* 132:2333S-2335S.

10. Poirier, L.A. (2002) The effects of diet, genetics and chemicals on toxicity and aberrant DNA methylation: an introduction. *J Nutr* 132:2336S-2339S.

11. Zhu, W.G., Lakshmanan, R.R., Beal, M.D.,Otterson, G.A. (2001) DNA methyltransferase inhibition enhances apoptosis induced by histone deacetylase inhibitors. *Cancer Res* 61:1327-33.

12. Januchowski, R., Dabrowski, M., Ofori, H.,Jagodzinski, P.P. (2007) Trichostatin A down-regulate DNA methyltransferase 1 in Jurkat T cells. *Cancer Lett* 246:313-7.

13. de Ruijter, A.J., van Gennip, A.H., Caron, H.N., Kemp, S.,van Kuilenburg, A.B. (2003) Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J* 370:737-49.

14. Adcock, I.M., Ford, P., Barnes, P.J.,Ito, K. (2006) Epigenetics and airways disease. *Respir Res* 7:1-21.

15. Verdin, E., Dequiedt, F.,Kasler, H.G. (2003) Class II histone deacetylases: versatile regulators. *Trends Genet* 19:286-93.

16. Barnes, P.J., Adcock, I.M.,Ito, K. (2005) Histone acetylation and deacetylation: importance in inflammatory lung diseases. *Eur Respir J* 25:552-63.

17. Glozak, M.A., Sengupta, N., Zhang, X.,Seto, E. (2005) Acetylation and deacetylation of non-histone proteins. *Gene* 363:15-23.

18. Vigushin, D.M.,Coombes, R.C. (2002) Histone deacetylase inhibitors in cancer treatment. *Anticancer Drugs* 13:1-13.

19. Sternglanz, R.,Schindelin, H. (1999) Structure and mechanism of action of the histone acetyltransferase Gcn5 and similarity to other N-acetyltransferases. *Proc Natl Acad Sci U S A* 96:8807-8.

20. Zhang, Y., Fatima, N.,Dufau, M.L. (2005) Coordinated changes in DNA methylation and histone modifications regulate silencing/derepression of luteinizing hormone receptor gene transcription. *Mol Cell Biol* 25:7929-39.

21. Kishikawa, S., Ugai, H., Murata, T.,Yokoyama, K.K. (2002) Roles of histone acetylation in the Dnmt1 gene expression. *Nucleic Acids Res Suppl* 209-10.

22. Januchowski, R., Dabrowski, M., Ofori, H.,Jagodzinski, P.P. (2006) Trichostatin A down-regulate DNA methyltransferase 1 in Jurkat T cells. *Cancer Lett*.

23. Jaenisch, R.,Bird, A. (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 33 Suppl:245-54.

24. Huber, L.C., Gay, R.E.,Gay, S. (2006) Synovial activation. In: A Falus (Ed). *Immunogenomics and Human Disease* Wileys:299-325.

25. Ermann, J.,Fathman, C.G. (2001) Autoimmune diseases: genes, bugs and failed regulation. *Nat Immunol* 2:759-61.

26. Smith, J.B.,Haynes, M.K. (2002) Rheumatoid arthritis--a molecular understanding. *Ann Intern Med* 136:908-22.

27. Kim, Y.I., Logan, J.W., Mason, J.B.,Roubenoff, R. (1996) DNA hypomethylation in inflammatory arthritis: reversal with methotrexate. *J Lab Clin Med* 128:165-72.

28. Kroger, H., Dietrich, A., Gratz, R., Wild, A.,Ehrlich, W. (1999) The effect of tryptophan plus methionine, 5-azacytidine, and methotrexate on adjuvant arthritis of rat. *Gen Pharmacol* 33:195-201.

29. Waterland, R.A. (2006) Assessing the effects of high methionine intake on DNA methylation. *J Nutr* 136:1706S-1710S.

30. Schwab, J.,Illges, H. (2001) Silencing of CD21 expression in synovial lymphocytes is independent of methylation of the CD21 promoter CpG island. *Rheumatol Int* 20:133-7.

31. Muller-Ladner, U., Kriegsmann, J., Franklin, B.N., Matsumoto, S., Geiler, T., Gay, R.E.,Gay, S. (1996) Synovial fibroblasts of patients with rheumatoid arthritis attach to and invade normal human cartilage when engrafted into SCID mice. *Am J Pathol* 149:1607-15.

32. Neidhart, M., Rethage, J., Kuchen, S., Kunzler, P., Crowl, R.M., Billingham, M.E., Gay, R.E.,Gay, S. (2000) Retrotransposable L1 elements expressed in rheumatoid

arthritis synovial tissue: association with genomic DNA hypomethylation and influence on gene expression. *Arthritis Rheum* 43:2634-47.

33. Kuchen, S., Seemayer, C.A., Rethage, J., von Knoch, R., Kuenzler, P., Beat, A.M., Gay, R.E., Gay, S.,Neidhart, M. (2004) The L1 retroelement-related p40 protein induces p38delta MAP kinase. *Autoimmunity* 37:57-65.

34. Ito, K., Ito, M., Elliott, W.M., Cosio, B., Caramori, G., Kon, O.M., Barczyk, A., Hayashi, S., Adcock, I.M., Hogg, J.C.,Barnes, P.J. (2005) Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N Engl J Med* 352:1967-76.

35. Huber, L.C., Hemmatazad, H., Brock, M., Moritz, F., Trenkmann, M., Distler, J.H., Gay, R.E., Simmen, B., Michel, B.A., Gay, S., Distler, O.,Jungel, A. (2007) Histone deacetylase/ acetylase activity in total synovial tissue derived from RA and OA patients. *Arthritis Rheum.* in press.

36. Chung, Y.L., Lee, M.Y., Wang, A.J.,Yao, L.F. (2003) A therapeutic strategy uses histone deacetylase inhibitors to modulate the expression of genes involved in the pathogenesis of rheumatoid arthritis. *Mol Ther* 8:707-17.

37. Blanchard, F.,Chipoy, C. (2005) Histone deacetylase inhibitors: new drugs for the treatment of inflammatory diseases? *Drug Discov Today* 10:197-204.

38. Ito, K., Yamamura, S., Essilfie-Quaye, S., Cosio, B., Ito, M., Barnes, P.J.,Adcock, I.M. (2006) Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-kappaB suppression. *J Exp Med* 203:7-13.

39. Jungel, A., Baresova, V., Ospelt, C., Simmen, B.R., Michel, B.A., Gay, R.E., Gay, S., Seemayer, C.A.,Neidhart, M. (2006) Trichostatin A sensitises rheumatoid arthritis synovial fibroblasts for TRAIL-induced apoptosis. *Ann Rheum Dis* 65:910-2.

40. Floess, S., Freyer, J., Siewert, C., Baron, U., Olek, S., Polansky, J., Schlawe, K., Chang, H.D., Bopp, T., Schmitt, E., Klein-Hessling, S., Serfling, E., Hamann,

A.,Huehn, J. (2007) Epigenetic control of the foxp3 locus in regulatory T cells. *PLoS Biol* 5:e38.

41.   Huber, L.C., Distler, O., Tarner, I., Gay, R.E., Gay, S.,Pap, T. (2006) Synovial fibroblasts: key players in rheumatoid arthritis. *Rheumatology (Oxford)* 45:669-75.

42.   Lu, Q., Qiu, X., Hu, N., Wen, H., Su, Y.,Richardson, B.C. (2006) Epigenetics, disease, and therapeutic interventions. *Ageing Res Rev* 5:449-67.

43.   Ou, J.N., Torrisani, J., Unterberger, A., Provencal, N., Shikimi, K., Karimi, M., Ekstrom, T.J.,Szyf, M. (2007) Histone deacetylase inhibitor Trichostatin A induces global and gene-specific DNA demethylation in human cancer cell lines. *Biochem Pharmacol* Epub ahead of print.

44.   Myzak, M.C., Ho, E.,Dashwood, R.H. (2006) Dietary agents as histone deacetylase inhibitors. *Mol Carcinog* 45:443-6.

45.   Dashwood, R.H., Myzak, M.C.,Ho, E. (2006) Dietary HDAC inhibitors: time to rethink weak ligands in cancer chemoprevention? *Carcinogenesis* 27:344-9.

46.   Feil, R. (2006) Environmental and nutritional effects on the epigenetic regulation of genes. *Mutat Res* 600:46-57.

47.   Roth, S.Y., Denu, J.M.,Allis, C.D. (2001) Histone acetyltransferases. *Annu Rev Biochem* 70:81-120.

6. FIGURE LEGENDS

Figure 1

Interaction between genes (genom), epigenetics (epigenom) and phenotype (adapted and modified after [46]). Postsynthetical modifications of DNA are inherited (as epimutations) or establish during development and cell differentiation. Physiological and pathological responses to environmental stimuli (such as nutrition, age, and infections) are also governed by epigenetic mechanisms. Even in the absence of such environmental factors, the epigenomic profile is reversible and highly variable, probably due to stochastic events in the somatic inheritance and in maintenance of epigenetic profiles.

Figure 2

The dynamic balance between silent and transcriptionally active chromatin (modified after [23]). Histone deacetylases (HDACs), DNA methyltransferases (DNMTs) and Methyl-CpG-binding proteins (MBPs) provide gene repression, whereas transcription factors (TFs), histone acetyltransferases (HATs) and HDAC inhibitors (HDACi) lead to enhanced gene transcription rates. Disturbances and changes in one or more of these components shift the balance to any side of gene expression.

Table 1

Histone acetyltransferase (HAT) superfamily (modified after [47]).
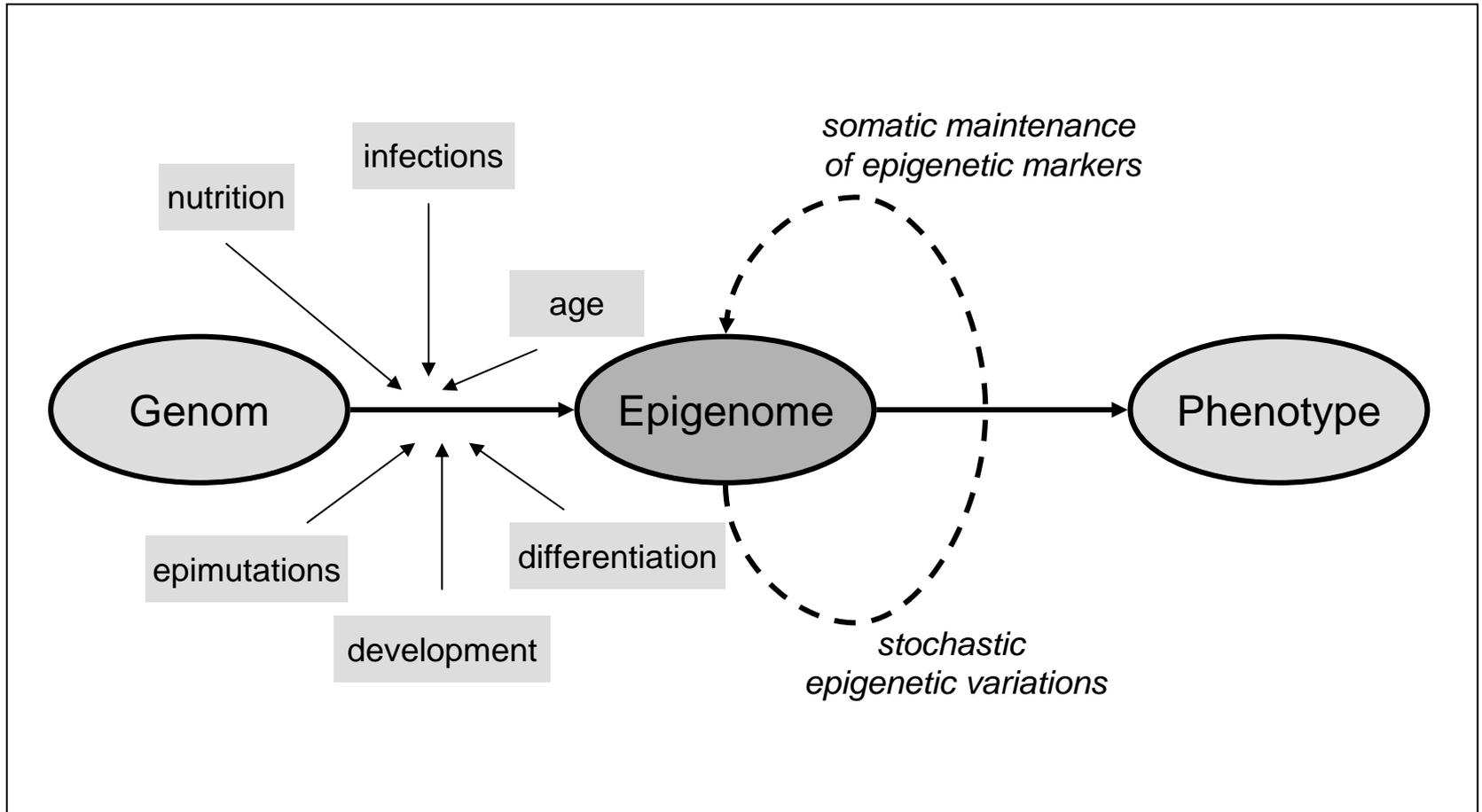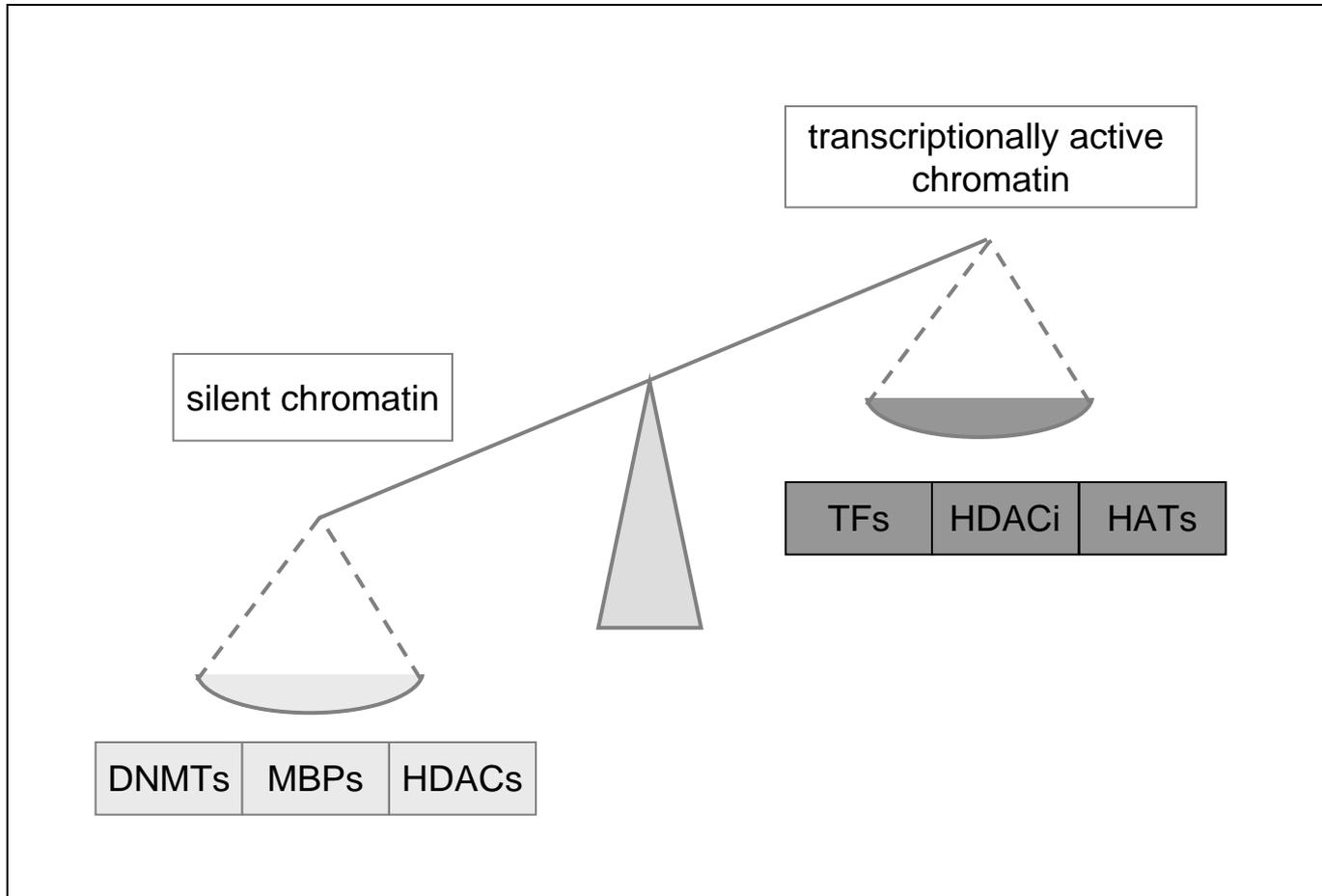
Figure 1

Figure 2

Table 1

HAT superfamily	HAT	Transcription-related functions	Histones acetylated	Interaction with other HATs
GNAT	Gcn5	Co-Activator	H3/H4	p300; CBP
	Hat1		H4	
	PCAF	Co-Activator	H3/H4	p300; CBP
	Elp3	Transcript elongation		
	Hpa2		H3/H4	
MYST	Esa1	Cell cycle progression	H4/H3/H2A	
	MOF		H4/H3/H2A	
	Sas2	Silencing		
	Sas3	Silencing	H3/H4/H2A	
	MORF		H4/H3/H2A	
	Tip60	HIV Tat interaction	H4/H3/H2A	
	Hbo1	ORC interaction		
p300/ CBP	P300	Global co-activator	H2A/H2B/H3/H4	PCAF; Gcn5
	CBP		H2A/H2B/H3/H4	PCAF; Gcn5