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Trenkmann, M; Brock, M; Ospelt, C; Gay, S (2010). Epigenetics in Rheumatoid Arthritis. Clinical Reviews in Allergy and Immunology, 39(1):10-19. Postprint available at:
http://www.zora.uzh.ch

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http://www.zora.uzh.ch

Originally published at:
Clinical Reviews in Allergy and Immunology 2010, 39(1):10-19.
Epigenetics in rheumatoid arthritis

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Abstract

Epigenetics is a steadily growing research area. In many human diseases, especially in cancers, but also in autoimmune diseases, epigenetic aberrations have been found. Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation and destruction of synovial joints. Even though the aetiology is not yet fully understood, rheumatoid arthritis is generally considered to be caused by a combination of genetic predisposition, deregulated immunomodulation and environmental influences. To gain a better understanding of this disease researchers have become interested in studying epigenetic changes in rheumatoid arthritis. Here we want to review the current knowledge on epigenetics in rheumatoid arthritis.
**Introduction**

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the joints affecting ~1% of the population worldwide [1]. One of the main characteristics of this autoimmune-related disorder is the thickened and hyperplastic synovial lining composed of infiltrating inflammatory cells and synovial fibroblasts (SF) [2]. Beside cytokines and chemokines that fuel the synovial inflammation these resident cells produce matrix degrading enzymes such as metalloproteinases which eventually leads to a progressive destruction of articular cartilage and bone [3]. Although the understanding of the cause of RA remains fractional it is generally accepted that it arises from an interplay of genetic predisposition (in particular HLA-DR allele subtypes and specific gene polymorphisms), immunological deregulation (e.g. autoantibody production) and environmental factors (such as nutrition or exposure to infectious agents) [4, 5].

Epigenetics is a novel area of research in rheumatic diseases. Depending on the interpretation of the definition of epigenetics, several perceptions of what epigenetics is can be found in the literature [6]. The classical view defines epigenetics as heritable changes in gene expression patterns that are not caused by changes in the primary DNA sequence. Today this definition has broadened to additionally include transient changes in gene expression [7].

Epigenetic regulation has a crucial role in development and differentiation – although all cells of an organism have the same DNA sequence they can differentiate into a multitude of diverse cell types [8]. DNA inside a eukaryotic cell is wrapped around an octamer of the core histones H2A, H2B, H3 and H4 thus building the fundamental unit of chromatin, the nucleosome [9]. Posttranslational modifications of the protruding histone tails and cytosine methylation of the DNA determine the accessibility of the chromatin and therefore the ability of transcription factors to bind and initiate gene expression [10] (Fig. 1). These chromatin marks, however, are frequently not stable; in fact, they can rapidly change in response to a stimulus [6, 9].
Failure of this system can lead to developmental defects and disease. Remarkably, the influence of environmental factors and aging on the epigenome has attracted attention as a possible explanation for age-related autoimmune diseases [7]. It is becoming increasingly clear that epigenetics play an important role in shaping the immune system and inflammatory response [11-13]. Therefore the study of epigenetics might help to understand why some genetically predisposed individuals develop rheumatoid arthritis while others do not, why some patients respond to presently available medication and others do not or how chronic inflammation and joint destruction are sustained. Here we want to assemble the current knowledge on epigenetics in rheumatoid arthritis and what we could learn from other disciplines for future research in this interesting new field.

**Histone modifications**

Histones are small globular proteins with flexible N-terminal tails that project from the nucleosome and hence are available for molecular interactions. These tails can be extensively modified – more than 60 different modification positions on histones have been found [9] – which is decisive for specific interrelations. The established histone modifications comprise acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, ADP ribosylation, deimination and proline isomerisation [reviewed in 9] of which acetylation and methylation are the most intensively studied. Histone tail modifications determine the strength of the DNA-histone and histone-histone interactions within and between nucleosomes. Thereby the higher-order chromatin structure is modulated, i.e. condensation and packaging or decondensation and unpacking of chromatin [14]. One histone can carry multiple histone modifications at a given time, and these modifications can influence each other. It has been suggested that the combination of histone modifications can be “read” like a code by specific binding proteins thereby leading to a very distinct transcriptional output [15].
Histone acetylation is generally associated with regions of actively transcribed chromatin. The addition of an acetyl group to lysine residues within the histone tail neutralizes their positive charge thereby disrupting the interaction with the negatively charged DNA which loosens up the chromatin structure. In addition, transcriptional activators are recruited by these acetyl-lysines via bromodomains and enhance gene activity [16]. The enzymes adding and removing acetyl groups from lysine residues in the histone tails are histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively [17, 18]. In contrast to acetylation, methylation of histone residues can either activate or repress gene expression. Methylation of histone 3 lysine 4, 36 and 79 (H3K4, H3K36 and H3K79) is found at active genes, methylation of histone 3 lysine 9 and 27 and histone 4 lysine 20 (H3K9, H3K27 and H4K20) at transcriptionally repressed genes [9]. Histone lysine methyltransferases catalyze the addition of up to three methyl groups in a site-specific manner, i.e., these enzymes can only recognize lysine residues within a very specific sequence of amino acids. Accordingly, also histone lysine demethylases act site-specific. The methylation of arginines is catalyzed by protein arginine methyltransferases (PRMTs). Up to now only one report of a histone arginine demethylases exists [19]. However, removal of the methyl group can also occur by demethylimination by peptidylarginine deiminase 4 (PAD4). Thereby the residue is converted to citrulline [20]. Interestingly, many histone modifying enzymes have been found to have non-histone protein substrates as well [21, 22].

An important role of epigenetic regulation of the immune system has been found in the differentiation of T helper (T$_{H}$) cells [11]. Whereas in naïve T cells almost no histone acetylation at the interferon-γ (Ifng) promoter or the interleukin(II) 4-Il13 locus was detected, cells acquired strong acetylation at and therefore transcription from the Ifng promoter upon differentiation to the T$_{H}$1 phenotype. By contrast T$_{H}$2 differentiated cells showed hyperacetylation and transcription of the Il4-I13 locus [23, 24]. Koyanagi and co-workers analyzed the involvement of histone methylation in the silencing of the Il4-I13 locus in T$_{H}$1
cells [25]. By chromatin immunoprecipitation they could show that in T₁₇₁ primed cells IL4 and IL13 get silenced by increased H3K27me3, a repressive histone mark, whereas T₁₇₂ primed cells did not exhibit such a modification at the IL4-IL13 locus and therefore had active transcription of these genes. Similarly, T₁₇₂ cells were shown to accumulate the H3K27me3 mark at the IFNγ promoter which was not found in T₁₇₁ cells [26]. RA patients are considered to have a T₁₇₁ biased phenotype [1]. Surprisingly, a report by Raza and colleagues showed high synovial fluid expression of IL-4 and IL-13 in early RA patients and very low expression in established RA implicating a temporary T₁₇₂ disposition in early RA [27]. Further studies are needed to find out if and how the epigenetically regulated T helper cell differentiation contributes to the transition from early to established RA.

Smoking is associated with an increased risk for developing RA and a more severe disease outcome [28]. A study by Yang et al. showed that chronic exposure to cigarette smoke led to strongly reduced expression of the histone deacetylases SIRT1 and 2 in the lungs of rats [29]. Furthermore, pre-treatment of macrophage cells with the SIRT1 activator resveratrol significantly inhibited the smoke-induced production of proinflammatory cytokines. Another member of the sirtuin family, SIRT6, was found to physically interact with the nuclear factor-κB (NF-κB) subunit RelA and repress NF-κB dependent gene expression by deactylating H3K9 [30]. Loss of SIRT6 resulted in resistance to Fas-associated protein with death domain (FADD)-induced apoptosis and hyperactivity of NF-κB target genes related to immune responses, cell signalling and metabolism [30]. On the other hand the production of tumor necrosis factor α (TNF-α), which is an inducer of NF-κB signalling, was found to be positively regulated by SIRT6 [31]. Whether these apparently conflicting functions of SIRT6 are part of a regulatory feedback loop or reflect cell type specific roles, still has to be elucidated. Coming back to RA, it would be intriguing to find connections between environmental factors such as smoking and epigenetic modulation of signalling pathways central to the immune system such as NF-κB signalling.
Studies of histone modifications in RA have mostly concentrated on histone acetylation and particularly on the use of HDAC inhibitors (HDI) as therapeutic agents. There have been a number of reports of beneficial effects from the use of HDI in experimental models of RA or in in vitro studies [32]. In a mouse model of autoantibody-mediated arthritis (AMA) intravenous application of the HDI FK-228 rapidly reduced the symptoms of arthritis with a marked decrease in the expression of TNF-α and IL-1β in the synovium [33]. Furthermore, the expression of the cell cycle inhibitors p16\(^{INK4a}\) and p21\(^{WAF1/Cip1}\) was increased, which could be linked to an increased protein acetylation at the p16\(^{INK4a}\) promoter after incubation of RA-FLS with the HDI in vitro. In the collagen-induced arthritis (CIA) model in mice and rats prophylactic effects of the HDIs SAHA and MS-275 could be demonstrated with significantly reduced arthritis scores [34]. Additionally, mice pre-treated with HDIs before the induction of arthritis showed reduced histologic scores and decreased levels of IL-6 and IL-1β in the serum. Further on a therapeutic application of MS-275 in rat CIA had beneficial effects on arthritis score, radiologic score and bone resorption [34]. Nakamura and colleagues examined the action of FK-228 on bone destruction in rat adjuvant-induced arthritis (AIA) and the generation of osteoclasts [35], which are considered key players in the process of bone erosion in RA [36]. In vitro FK-228 inhibited the differentiation of osteoclasts, whereas in vivo it suppressed the development of AIA; nevertheless, treatment of AIA with FK-228 did not reduce the arthritis score although a significant reduction in bone erosions was observed [35]. Trichostatin A (TSA), yet another HDI, was shown to reduce the clinical scores of arthritis and synovial inflammation in mouse AMA, which was accompanied by a reduction in the expression of matrix-degrading enzymes [37]. All these studies suggest HDIs as possible therapeutics for RA, which would result in a global decrease of HDAC activity. However, studying whole synovial tissue of RA patients Huber and co-workers demonstrated that the balance of HAT and HDAC activity is already shifted towards a loss of HDAC activity as compared to osteoarthritis (OA) and normal synovium [38]. In particular, the
expression of HDAC1 and 2 was significantly reduced. In the light of this paper it seems that HDIs might not be suitable for the treatment of RA. Still, it is not clear whether some HDACs are upregulated despite the global reduction in HDAC activity and whether they could be targeted by certain HDIs, which usually show a moderate selectivity for distinct HDACs or HDAC classes [39]. Also, the exact expression pattern of HDACs in the different synovial cell types has not been studied and so it can not be precluded that HDAC activity/expression might be elevated in some cell types and therefore be targeted by HDIs in the animal models. Nevertheless, it must be kept in mind that our knowledge on how the beneficial effects of HDIs come about is very limited and also that these effects might be different between mice and men. Since HDACs have many non-histone protein targets it is still open to which degrees chromatin remodelling and acetylation/deacetylation of other cellular proteins are at work. Therefore further research is inevitable.

Of the four known peptidylarginine deiminases (PAD) that deiminate arginine residues in proteins to generate citrulline only PAD4 (formerly known as PAD5) was shown to be localized to the nucleus and to be capable of deiminating/demethyliminating histones [20, 40]. Autoantibodies against citrullinated proteins (anti-CCP) are very specific for RA and can be found in about 80% of the patients [41]. Therefore studying the citrullination of proteins is of major interest in RA. PAD4 is expressed in different cell types of the RA synovium as well as in blood monocytes [42, 43] and recently Chang and co-workers reported increased expression of PAD4 in RA synovial membranes compared to OA [44]. However, it is still unclear to what extent PAD4 contributes to epigenetic changes involved in the pathogenesis of RA. In granulocytes differentiated from the neutrophil cell line HL-60 PAD4 is required for the hypercitrullination of histones, and blood neutrophils stimulated with proinflammatory stimuli showed a rapid increase in histone citrullination which led to an extensive chromatin decondensation [45, 46]. Such decondensed chromatin can be secreted from neutrophils as an innate immune response. Neeli and colleagues put the idea forward that this release of a
potential autoantigen into the extracellular space might represent the link between an initial infection and the development of autoimmunity [46]. Whether there is increased arginine deimination/demethylimination and hypercitrullination of histones in RA is not known, therefore this hypothesis needs further experimental substantiation.

DNA methylation

In contrast to the variety of histone modifications, methylation represents the only known physiologic alteration of the chemical composition of DNA. In prokaryotes the addition of methyl groups occurs at adenosine and cytosine bases, whereas in multicellular eukaryotes methylation is defined to cytosine bases within CpG dinucleotides [47]. Such CpG sequences are clustered in so called CpG islands and are mainly located in 5’ regulatory sites of genes leading to an unusually rich GC content of DNA. In general, the methylation process is catalyzed by the protein family of DNA methyltransferases (DNMT), which uses the methyl donor S-adenosylmethionine (SAM) to specifically methylate the fifth carbon atom of the cytosine ring [48]. In regard to their physiological function, the mammalian DNMTs can be grouped into 2 different classes. DNMT3A and DNMT3B act as de novo methyltransferases by introducing methyl groups into previously unmethylated CpG dinucleotides. Maintenance of the DNA methylation pattern during cell division is achieved by DNMT1, which recognizes established methylation marks and copies them to the newly synthesized DNA strand [47].

It is commonly accepted that DNA methylation causes transcriptional silencing of associated genes. There are two fundamental mechanisms by which transcription of methylated DNA can be suppressed. On the one hand methylation of cytosine bases directly decreases the affinity for binding of transcription factors and on the other hand methyl groups recruit methyl-CpG-binding proteins (MBPs) to promoter regions where they associate with classical co-repressors leading to an impaired transcriptional process [49]. There is an increasing body
of evidence suggesting a pivotal role for DNA methylation in the pathogenesis of human disease as well as during normal biological processes. For instance, it was shown that in resting naïve T cells the expression of IL-2 is silenced by DNA methylation of the promoter region. When T cells are activated by T cell receptor dependent stimulation, the IL-2 promoter becomes rapidly demethylated and thus production of IL-2 is induced [50]. Recently it was reported that the expression of another potent cytokine, TNF-α, is regulated by epigenetic mechanisms as well [51]. Sullivan and co-workers investigated DNA methylation of CpG rich sequences within the promoter of the TNF gene and found a positive correlation between high expression levels of TNF-α and low methylation status. Since cytokines are involved in many aspects of the pathogenesis of RA [52] it would be worthwhile to find out whether their expression in RA is affected by deviant DNA methylation.

A first hint that patients suffering from RA exhibit an aberrant DNA methylation pattern was given by Richardson et al. [53]. By analyzing DNA methylation in T cells they found that the global methylation of genomic DNA of T cells derived from RA patients was less than of T cells from healthy controls. The phenomenon of hypomethylated genomic DNA in RA was also discussed by Neidhart et al. [54]. The authors identified the presence of the retrotransposable element LINE1 in RA synovial fluid pellet. Retrotransposons are retroviral-like mobile genetic elements and are involved in various diseases. Since retrotransposons have the ability to integrate into host genomes, they can affect gene expression leading to an impaired cellular phenotype. As Neidhart et al. could further show, the expression of LINE1 was significantly enhanced in cultured FLS derived from RA patients. As retrotransposons are normally regulated by DNA methylation, they concluded that this might be connected to the phenomenon of globally hypomethylated DNA in RA [54].

An altered pattern of methylation of genomic DNA in RA was reported in a study published by Nile and co-authors [55]. They examined the methylation status of CpG dinucleotides in the 5’ regulatory region of the IL-6 gene in peripheral blood mononuclear cells (PBMCs) and
found a single unmethylated CpG motif in RA patients when compared to healthy donors. Furthermore, by employing EMSA experiments Nile et al. gave evidence that enforced methylation of this particular CpG site prevented the binding of nuclear proteins to the genomic DNA. Based on their findings the authors proposed that hypomethylation contributes to an elevated expression of the IL-6 gene by supporting the binding of nuclear proteins. Considering the fact that global hypomethylation of genomic DNA has been identified as a participant in the aggressive behaviour of cancer cells by the upregulation of oncogenes such as c-Myc or h-Ras [reviewed in 56] one could speculate that DNA hypomethylation might contribute to the activated phenotype of RASF, which includes increased resistance against apoptosis, an altered response to inflammatory stimuli, and an increased production of matrix-degrading enzymes [3]. However, the impact of genomic hypomethylation on the pathogenesis of RA needs to be defined more clearly by further studies.

Despite global hypomethylation, distinct CpG islands located in regulatory regions of genes can be specifically hypermethylated leading to a repressed transcription of the associated genes [56]. Up to now, numerous genes have been identified in cancer that are silenced by hypermethylation, including genes involved in cell cycle control (e.g. CDKN2, [57]), apoptosis (e.g. DAPK1, [58]), DNA repair (e.g. BRCA-1, [59]), metastasis (e.g. TIMP-3, [60]), drug resistance, differentiation, and angiogenesis. Thus, methylation of CpG dinucleotides is a common biological mechanism for switching off gene expression, and more interestingly, this is not only limited to cancer. Local hypermethylation of CpG islands was also reported in autoimmune diseases such as rheumatoid arthritis. The death receptor 3 (DR3), a member of the apoptosis-inducing Fas family, was found to be expressed to a lesser extent in synovial cells derived from RA patients when compared to patients suffering from OA [61]. By studying methylation of the CpG island surrounding the transcription start site of DR3 the authors found a significant hypermethylation of CpG dinucleotides in synovial cells derived from RA, which might explain the impaired expression of DR3. Since DR3 can
trigger the intrinsic apoptosis machinery [62] the findings of Takami and co-workers provide a novel link between altered DNA methylation and resistance to apoptosis seen in synovial cells of RA patients.

**Non-coding RNAs**

Chromatin structure is not exclusively regulated by covalent modifications such as DNA methylation and histone modifications. Chromatin dynamics are also influenced by chromatin-remodelling complexes - big ATP consuming machines that reposition nucleosomes, interfere with the DNA-histone interactions or facilitate histone variant replacement [63]. Other relevant regulators of chromatin structure and gene expression are non-coding RNAs (ncRNAs, i.e. transcripts that are not translated into protein) which can range in size from a few nucleotides (nt) to several kilobases (kb) [64]. A prominent member of the large ncRNAs is the *Xist* RNA which is important for X chromosome inactivation. In mammals, females have two copies of the X chromosome of which one needs to be silenced to obtain dosage compensation in comparison to males [65]. *Xist* is transcribed from the so called X inactivation centre (Xic) of the future inactive X chromosome (Xi) and initiates silencing by coating the surrounding territory of the chromosome [66]. Stable silencing is then accomplished by the addition of repressive histone marks, DNA methylation and accumulation of the histone variant macroH2A [67]. Since women have a higher prevalence for a variety of autoimmune-related disorders than men, X chromosome inactivation has been implicated in these diseases [68]. Studies in systemic lupus erythematosus (SLE) and systemic sclerosis indeed point to a skewed X inactivation [69, 70]. Lu and co-workers showed that *CD40LG*, which is located on the X chromosome, is overexpressed in CD4+ T cells from women with SLE, and this overexpression correlated with reduced DNA methylation of its promoter region [69]. However, no such study has yet concentrated on the role of the *Xist* RNA in the induction of Xi. Also, Xi has not yet been investigated in the context of RA. The
future will show whether defective X inactivation – locally or globally – can be identified in RA and which role ncRNAs might play in this process.

Small ncRNAs can mediate both transcriptional and post-transcriptional gene silencing (TGS and PTGS) [71]. Endogenous short interfering RNAs (siRNAs, 20-25 nt in size) were found to be involved in TGS by targeting chromatin-modifications to promoter regions; however, their precise role and mechanism of action in mammalian cells is not yet well understood [72]. MicroRNAs (miRNAs) are a group of small, ncRNA molecules (18 – 22 nt) that function as post-transcriptional regulators of gene expression. These ribonucleotides were initially identified in *C. elegans* at the beginning of the last decade [73]. It is now evident that miRNAs are widely expressed in plants, animals and men [74]. All human miRNAs examined so far are transcribed in the nucleus by the action of RNA polymerase II or III forming long preliminary transcripts [75]. Subsequently, these precursors are cleaved by two RNase type III-endonucleases: first by Drosha in the nucleus and then after export to the cytoplasm by DICER. The resulting mature miRNAs bind to their target mRNAs by base pairing at distinct regions, and thus alter mRNA stability or affect protein translation [76]. It is generally accepted that miRNAs preferentially bind to complementary sites located in the 3’UTR of target mRNAs and that the degree of complementarity determines how the target will be repressed: perfect complementarity leads to mRNA cleavage whereas partial binding induces repression of translation [77]. Whichever way, repression is always mediated by the RNA-induced silencing complex (RISC) which is shared by the miRNA and siRNA pathways alike. Computational methods predict that up to one third of the human transcripts are regulated by miRNAs [78]. Consequently, miRNAs have been associated with numerous biological processes including cell cycle regulation (e.g. [79]) apoptosis (e.g. [80]) and differentiation (e.g. [81]). One of the first studies reporting an involvement of miRNAs in the inflammatory response demonstrated the upregulation of miRNA 155 (miR-155) in murine macrophages in response to Toll-like receptor ligands [82]. Furthermore, the authors gave evidence that the
transcriptional activation of the miR-155 gene was, at least in part, achieved by the activation of the mitogen-activated protein kinase (MAPK) pathway. Toll-like receptors (TLRs) are part of the pattern-recognition system of the innate immune system and represent the first line of host defence against pathogens. Interestingly, TLRs were found to be overexpressed in the synovial tissue and in synovial cells of RA reflecting the chronic inflammatory milieu of the disease [83]. Consequently, in the first paper on miRNAs in RA, elevated expression of miR-155 in cultured synovial cells from RA patients as well as in CD14+ monocytes/macrophages was reported [84]. Furthermore, increased levels of miR-155 after exposure to TNF-α, IL-1β or TLR ligands were detected. With respect to the data presented by O'Connell et al. one could conclude that miR-155 is an inflammatory marker because of its induced expression after inflammatory stimuli. Nevertheless, despite these findings little is known about the function of miR-155 in RA. Stanczyk and co-workers investigated the effect of enforced expression of miR-155 on the production of matrix-metalloproteinase 3 (MMP3) in synovial cells and found significant repression of MMP3 when compared to scrambled negative control [84]. These authors suggested that miR-155 might act as a protective miRNA by repressing the expression of certain MMPs. Interestingly, Ceppi et al. recently demonstrated that miR-155 directly targets TAB2, a signal transduction molecule that mediates the activation of the inflammatory response upon IL-1β and TLR4 stimulation [85]. In conclusion, miR-155 evolves as a new player in the inflammatory network that seems to be upregulated upon cytokine stimulation and to negatively regulate inflammatory gene expression.

In concert with the findings presented by Stanczyk et al. elevated expression of miR-155 and miR-146 in RA was demonstrated by two further studies [86, 87]. Nakasa and co-workers found that miR-146 was upregulated in RA synovial tissue and that its expression in RA-SF was induced by stimulation with TNF-α and IL-1β. However, the function of this miRNA in RA was not explored by these authors. Pauley et al. showed elevated miR-146 levels in
PBMCs from RA patients. From other studies, it is known that miR-146 functions as a negative regulator of the NF-κB pathway in human monocytes [88]. Therefore one could speculate that miR-146 plays a role in the fine-tuning of cytokine signalling in RA as well. Interestingly, Pauley et al. demonstrated that this feedback regulation mediated by miR-146 is impaired in PBMCs derived from RA and, thus, might explain the prolonged production of cytokines such as TNF-α in RA [87].

Although a detailed function is only known for very few miRNAs, it is obvious that these tiny RNA molecules influence the expression of hundreds of genes [89]. Further studies are needed to illuminate the mechanisms of miRNA biogenesis and action. Only specific insights into local and global expression patterns of miRNAs can help to understand how they modulate gene regulation in normal conditions and, eventually in disease.

**Outlook**

Knowledge on epigenetic processes in the setting of human diseases is rapidly increasing [7]. Great efforts are now undertaken to elucidate the role of epigenetic changes in the pathogenesis of autoimmune disorders such as rheumatoid arthritis. Although we do not have a general picture of how epigenetics influence this disease, some parts of the puzzle are beginning to take shape. What we need now are more detailed studies of how certain disease related genes are epigenetically regulated and how certain environmental or internal influences might have contributed to such changed epigenotypes. In this regard the investigation of the discordance between monozygotic twins will be of special interest. On the basis of their genetic sameness monozygotic twins were found to accumulate epigenetic differences during their lifetime which was on the one hand attributed to external factors such as smoking or diet. On the other hand an aging associated “epigenetic drift” (faulty transfer of epigenetic information during successive cell divisions) was proposed as an explanation [90]. If one of the monozygotic twins already suffers from the disease, the risk for the second twin
to develop RA is 15% [91], which clearly points to a strong non-genetic component in the pathogenesis of the disease and epigenetics might be one of these factors.

Another obvious question that remains is that of “the chicken and the egg” [38] – do the epigenetic changes come first and then the disease or is it the other way around? Is there a requirement for certain epimutations for rheumatoid arthritis to develop or are epigenetic changes acquired during the disease course due to the permanent activation of the immune system? In all probability the truth lies somewhere between these two scenarios. Some epigenetic changes in the immune system and/or the joints might favour the development of RA, and in the established disease chronic inflammation could keep up acquired epigenetic changes which in turn participate in maintaining inflammation and destruction of the joint (Fig. 2). Regarding the success of cytokine targeting therapies in RA (such as TNF-α blockade) this cytokine-driven self-fuelling process might be disrupted together with other non-epigenetic processes. In non-responders (these are e.g. up to one third of the patients receiving TNF-α blockers), blocking of a single cytokine does not seem to be enough to alleviate the symptoms. Furthermore, in many cases the symptoms return when therapy is stopped. This could be due to inflammation-independent processes or an epigenetically imprinted disease phenotype. Nevertheless, epigenetic therapies – despite the demonstrated beneficial effects of HDIs in arthritis animal models – do not seem to be an option at the moment. We still do not know enough about how epigenetic marks work in detail and to which extent they are involved in RA. In this respect, adverse reactions are most likely whose magnitude cannot be estimated. Still, it is exiting to monitor how our knowledge of epigenetics in general and in human diseases is going to increase in the coming years and how this will help to find answers for yet unsolved questions in RA.
**Figure legends**

Figure 1:
Epigenetic modifications in transcriptionally active and repressed chromatin

Distinct posttranslational modifications (PTMs) of the histones can either be associated with open or closed chromatin, whereas DNA methylation generally functions to repress gene expression. This static picture, however, is not complete. Transcription is rather regulated by dynamic chromatin marks. Therefore the transcriptional output is context-dependent (the combination of histone PTMs, the positioning of certain PTMs inside the gene or during the different stages of transcription), and a histone PTM that is traditionally considered to be activating can also be found in repressed genes [10]. Ac, acetylation; H, histone; K, lysine; Me/me, methylation; P, phosphorylation; SUMO, sumoylation; Ub, ubiquitylation.

Figure 2:
Model for the interplay of epigenetics with other factors in the pathogenesis of rheumatoid arthritis

17
References


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Figure 1

Open chromatin

Co-activators binding to the chromatin

Closed chromatin

Co-repressors binding to the chromatin
Figure 2

chronic inflammation

destruction of cartilage and bone

production of auto-antibodies

environmental factors

changes to the epigenome

long-lasting changes in gene expression

genetic predisposition

chronic inflammation