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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease leading to joint destruction. Synovial fibroblasts are recognized as key cells in the pathogenesis of RA since they attract and activate immune cells and produce matrix degrading enzymes. Most notably synovial fibroblasts from patients with RA are stably activated and produce high levels of disease-promoting molecules without further stimulation by immune cells. Accumulating data suggest that epigenetic changes in stromal cell populations might be crucially involved in the pathology of RA and other chronic inflammatory diseases. In the current review, we discuss the mechanisms by which epigenetic changes might cause the stable activation of synovial fibroblasts in RA and how changes in the epigenome might alter immune function and inflammatory response and thereby promote the development of chronic diseases.
INFLAMMATORY MEMORIES: IS EPIGENETICS THE MISSING LINK TO PERSISTENT STROMAL CELL ACTIVATION IN RHEUMATOID ARTHRITIS?

Caroline Ospelt\textsuperscript{a,b}, Kris A. Reedquist\textsuperscript{b}, Steffen Gay\textsuperscript{a}, Paul P. Tak\textsuperscript{b}

\textsuperscript{a} Center of Experimental Rheumatology, University Hospital Zürich, Switzerland

\textsuperscript{b} Division of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, The Netherlands

Corresponding author:

Caroline Ospelt
Center of Experimental Rheumatology
University Hospital of Zürich
Gloriastrasse 23
CH-8091 Zürich
Switzerland
Tel.: +41 44 255 5866
Fax.: +41 44 255 4170

caroline.ospelt@usz.ch
ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disease leading to joint destruction. Synovial fibroblasts are recognized as key cells in the pathogenesis of RA since they attract and activate immune cells and produce matrix degrading enzymes. Most notably synovial fibroblasts from patients with RA are stably activated and produce high levels of disease-promoting molecules without further stimulation by immune cells. Accumulating data suggest that epigenetic changes in stromal cell populations might be crucially involved in the pathology of RA and other chronic inflammatory diseases. In the current review, we discuss the mechanisms by which epigenetic changes might cause the stable activation of synovial fibroblasts in RA and how changes in the epigenome might alter immune function and inflammatory response and thereby promote the development of chronic diseases.

Keywords: Epigenetics, synovial fibroblasts, rheumatoid arthritis
1. Introduction

In recent years, understanding of the pathogenesis of rheumatoid arthritis (RA) increased exponentially, leading to the development of novel targeted therapies as well as to changes in general treatment strategies. At the same time however, it became clear that the pathogenesis of RA is highly complex. Studies showing the presence of auto-antibodies and increased levels of cytokines and chemokines years before the first clinical symptoms clearly demonstrated that the disease is already far advanced when joint destruction starts and increased the awareness that even at the onset of symptoms irreversible damage of articular structures is imminent [1, 2]. It is now known that the genetic background, environmental influences, aberrant activation of the innate immune system as well as antigen-driven T and B cell responses are of importance. However it is not clear how and whether these different aspects are linked to cause disease. Furthermore, it still unknown why the synovium is primarily affected and which factors lead to the pathognomonic symmetric localization of joint involvement.

Up to now two distinct subsets of RA are discerned, namely RA positive for anti-citrullinated peptide antibodies (ACPA) and ACPA negative disease. These RA subtypes do not only differ in their clinical course but also in their underlying mechanisms. Smoking for instance has been shown to mainly increase the risk to develop ACPA positive RA [3]. Interestingly, this risk is much higher in individuals carrying shared epitope alleles providing evidence for a strong gene-environment interaction in this subgroup of patients [4, 5]. Apart from the shared epitope a variety of different allelic variants have been found to influence disease susceptibility and outcome alone or in combination, but direct connections between gene polymorphisms and pathogenetic pathways are elusive. Furthermore, most of the risk loci found up to now are not specific for RA and seem to generally increase the risk for autoimmunity pointing to additional factors triggering disease onset [6]. As recently highlighted by a special issue of the Journal of Autoimmunity, epidemiological studies gave
new insights which and how environmental factors might influence the development and the course of RA [7]. In particular, epigenetic changes could be identified as an important link between the environment and changes in gene expression.

Studies analysing the role of synovial fibroblasts in RA (RASFs) have significantly contributed to the overall understanding of local processes in joint destruction and chronic synovitis in RA, as RASFs not only strongly respond to a pro-inflammatory environment, but also actively and autonomously drive joint inflammation and destruction. They do so by producing a variety of pro-inflammatory cytokines, chemokines and matrix degrading enzymes. Most notably, RASFs appear to have a stably imprinted, activated phenotype when compared to healthy synovial fibroblasts. These features not only make RASFs an attractive target for future therapeutics but also give us the opportunity to learn more about local pathogenic pathways in RA.

2. Synovial fibroblasts: key cells of joint destruction in Rheumatoid Arthritis

2.1 Activation of RASFs

The pro-inflammatory environment in RA joints including high levels of cytokines, growth factors and infiltrating inflammatory cells strongly activates RASFs. The best studied activators of RASFs are TNF-α and IL-1 which are mainly produced by macrophages. In addition, RASFs express a variety of intra-and extracellular pattern-recognition receptors which allows them to react to environmental danger signals and make them important effector cells of the innate immune system. The Toll-like receptors (TLRs) 1-6, as well as the intracellular Nod-like receptor NOD2, are expressed and functional in RASFs and induce activation of NF-κB and mitogen-activated protein kinases (MAPKs) [8, 9]. Each of these pattern-recognition receptors recognizes different conserved pathogen-associated molecular patterns (PAMPs) derived from bacteria, virus, fungi or protozoa, and induces an immune response against these infectious agents. Even though an infectious trigger for the
development RA has not been found, bacterial DNA and peptidoglycans were shown to be retained in arthritic joints, which might lead to constant activation of TLR pathways [10]. Interestingly, TLRs were not only described to bind PAMPs but also to react to damage-associated molecular patterns (DAMPs) that are commonly increased after tissue injury. A variety of DAMPs have been found to be elevated in the joints of RA patients and via activation of TLRs might lead to perpetuation of the chronic inflammatory and destructive process. For instance, the extracellular matrix glycoprotein tenascin-C and the DNA binding protein HMGB1 are both elevated in RA and provoke a pro-inflammatory response via TLR signalling [11-13]. Also, double-stranded RNA from necrotic cells has been shown to induce the expression of IL-6 and MMPs in RASFs via TLR3 [14].

2.2 The imprinted phenotype of RASFs

RASFs not only strongly respond to a pro-inflammatory environment, they also actively and autonomously drive joint inflammation and destruction. RASFs appear to have a stably imprinted, activated phenotype when compared to healthy synovial fibroblasts [15]. This phenotype of RASFs is impressively demonstrated in experiments where RASFs are co-implanted with normal human cartilage under the kidney capsule of severe combined immunodeficient (SCID) mice. Isolated RASFs, despite several passages in culture, but not osteoarthritis (OASFs) or healthy synovial fibroblasts deeply invade the co-implanted cartilage in the absence of other inflammatory cells [16]. Continuing work with this model recently showed that RASFs can migrate via the bloodstream to implanted cartilage at a distant site [17]. This remarkable migratory potential of RASFs might partly explain the polyarticular affection which is typically found in RA patients. Even though overall evidence has been accumulated that synovial fibroblasts in RA differ in their phenotype from normal synovial fibroblasts and that these changes in behaviour are imprinted and stable over several passages in culture, the mechanisms behind this altered phenotype remain as yet elusive.
3. Epigenetics

3.1 Histone modifications and DNA methylation

Histone tails are modified by methylation, acetylation, phosphorylation, ubiquitinylation, SUMOylation and poly-ADP-ribosylation. All of these different histone modifications can be placed at various sites of the histone tails and together compose a complex histone code which confers transcriptional repression or activation for a specific genomic site [18]. Histone deacetylases (HDACs) are the group of enzymes that remove acetyl groups from histone tails which leads to condensation of chromatin structures and repression of gene expression. Counterregulatory to HDACs, histone acetyl transferases (HATs) acetylate histone tails. However, histones are not the only substrates for HDACs and HATs. Also transcription factors such as NF-κB or STATs are acetylated and deacetylated by HATs and HDACs. Deacetylation of STAT1 by HDAC3 for instance is a prerequisite for its phosphorylation and proper activation. This effect is reversed by the CREB binding protein (CBP)/p300 which acetylates STAT1 and thereby inhibits STAT1 signaling [19].

Modification of DNA by DNA methyltransferases (DNMTs) is an important mechanism to shape the gene expression pattern of a cell according to its environment. DNMTs add methyl groups to cytosines mainly at sites with a high content of guanine and cytosines, so-called CpG islands. Methylation of CpG islands in gene promoters leads to gene silencing via inhibition of transcription factor binding or via attraction of transcriptional repressors. During development, DNA methylation regulates the expression of tissue specific genes, represses the expression of repetitive sequences such as retrotransposons and mediates inactivation of the silent X chromosome in female cells. Loss of methylation in oncogenes and aberrant hypermethylation in tumor suppressor genes has been found to contribute to cancer growth and metastasis.
Strictly speaking epigenetic changes are changes in gene function that are heritable over generations without altering the DNA sequence. However short lived chromatin modifications can also substantially alter gene function and thereby indirectly initiate heritable epigenetic changes of the genome. Therefore the definition of epigenetics today is loosened to include not only classical epigenetic changes such as methylation of DNA and histone tails, but also more transient changes such as histone acetylation and phosphorylation [20]. Epigenetic mechanisms recently raised great interest in the study of the pathogenesis of chronic diseases. Since epigenetic changes seem to register and perpetuate alterations in cellular activity, they might not only explain lack of inflammatory resolution but also the connection between environmental risk factors and disease onset. The formation of an international epigenetic autoimmune group during the 10th International Symposium on Sjögren’s Syndrome in 2009 reflects the great interest that epigenetics also raised in the study of autoimmune diseases [21]. More and more evidence accumulates that histone modifications and DNA methylation can influence immune function and contribute to the development of autoimmunity. Modification of the epigenome have been found in systemic lupus erythematoses (SLE), multiple sclerosis, Sjögren’s Syndrome, systemic sclerosis and RA (compiled in [22]).

3.2 Histone modifications in RA

In RA, analysis of epigenetic changes is still in its early stages, but some interesting insights about how epigenetics might contribute to the pathogenesis of RA have already been provided. Changes in the expression and activity of histone deacetylases (HDACs), which in addition to modifying histone tails can negatively regulate gene promoter access, have been reported by Huber and colleagues. They observed decreased HDAC activity in RA synovial tissues compared to OA synovial tissues with decreased levels of HDAC1 and HDAC2, suggesting a global increase in histone and non-histone acetylation [23]. However, Kawabata et al have found that HDAC activity is higher in the synovial tissues of RA patients than in OA patients.
and HDAC1 expression was increased, which would lead to a deacetylated state in RA [24]. Although data is limited, it has been speculated that these differences might stem from differences in the medical treatment of the studied patients [25]. Up to now it is not clarified whether in RA the scale is tipped towards hyper- or hypoacetylation, but an immunohistochemical study with antibodies against acetyl-lysine showed strong acetylation in macrophages and RASFs in RA synovial tissues [25].

In contrast to uncertainties about potential changes in global histone acetylation, enhanced histone acetylation at promoters of specific genes relevant to pathology in RA has been observed. Changes in histone acetylation were linked to high levels of the small ubiquitin-related modifier SUMO-1 in RASFs. Histone acetylation in the matrix-metalloproteinase 1 (MMP1) promoter was increased in RASFs compared to OASFs, but could be normalized by enforced expression of the sentrin-specific protease SENP1 which cleaves SUMO-1 [26]. Accordingly, SENP1 overexpression downregulated expression levels of MMP-1. Since this effect was dependent on the expression of HDAC4 the authors hypothesized that increased SUMOylation of HDAC4 in RASFs prevents its nuclear translocation and subsequent deacetylation of histones at the MMP-1 promoter. Since acetylation of histones is a general mechanism that keeps the expression of genes turned on, it can be assumed that this mechanism does not only influence the expression of MMP-1 but might be responsible for the increased expression of a variety of other genes in RASFs.

Alternatively, strategies aimed at decreasing HDAC function may have therapeutic possibilities in RA, since administration of different HDAC inhibitors has shown promising results in arthritis animal models [27-29]. Also, in vitro treatment of RA macrophages and synovial explants showed potent anti-inflammatory actions of HDAC inhibitors [30]. The mechanisms by which HDAC inhibitors promote anti-inflammatory effects are not completely understood, but could stem from enhanced acetylation of histones at the promoters of
transcriptional repressors, enhanced transcription of anti-inflammatory gene products, or modification of non-histone targets [31].

3.3 Changes of DNA methylation in RA

Evidence of disturbed patterns of DNA methylation has been found in RA. Aberrant expression of the retrotransposon LINE-1 (long interspersed nucleotide elements–1) in RASF but not OASF suggests specific promoter demethylation in RA [32, 33]. Retrotransposons are mobile DNA sequences which can relocate within the genome, but are normally silenced by DNA methylation. The LINE-1 retrotransposon is one of the most common retrotransposons found in the human genome. Expression of LINE-1 elements in RASFs induces transcription of p38δ, galectin-3 binding protein, and the met protooncogene, demonstrating that the presence of functional LINE-1 can induce changes in gene expression [32]. The hypothesis that expression of LINE-1 in RASFs was due to loss of methylation marks in RA was corroborated by the finding that compared to OASFs or healthy synovial fibroblasts, genomic DNA of RASFs was globally hypomethylated [34]. Furthermore, it could be shown that while OASFs normally increased their levels of the DNA methyltransferase DNMT1 during proliferation, thereby assuring proper restoration of methyl marks in the newly synthesized DNA strand, proliferating RASFs showed a relative lack of DNMT1. This deficiency of DNMT1 might cause a loss of methylation marks in daughter cells, leading to progressive changes in the phenotype of RASFs over time.

Up to now only a few studies have analyzed and identified specific promoter regions that are differentially methylated in RASFs versus OASFs. The constitutively increased expression of CXCL12 by RASFs for instance, could be shown to be due to relative hypomethylation of its promoter region in RASFs compared to OASFs and healthy synovial fibroblasts [35]. High CXCL12 levels in turn stimulate production of MMPs, a mechanism that might partly be responsible for the destructive behaviour of RASFs in the SCID mouse co-implantation model.
and in human patients. In line with epigenetic mechanisms regulating CXCL12 production in RASFs, in RA patients treated with TNF blocking agents levels of CXCL12 remain high and a low grade disease activity persists, whereas after synovectomy CXCL12 is significantly reduced [36, 37].

Similar to findings in tumor cells, not only hypomethylation but also hypermethylated promoter regions were detected in RA. A specific CpG island in the promoter of death domain receptor 3 (DR-3) was shown to be hypermethylated in RASFs and accordingly expression of DR-3 was decreased in RASFs compared to OASFs [38]. Interestingly, also in PBMCs and T cells from RA patients disturbances of DNA methylation could be detected [39]. Particularly, in RA PBMCs loss of methylation in the promoter of the IL-6 and the ephrinB1 gene was found to be connected to increased expression of these genes in RA [40, 41].

It is not clear yet how broadly changes in DNA methylation patterns affect the phenotype of RASFs. In the human genome, primarily sequences outside of promoters are strongly methylated, while around 95% of CpG islands in promoter regions are demethylated [42]. The global hypomethylated state of DNA in RASFs would therefore mainly affect non-regulatory sequences such as retrotransposons and repetitive sequences, coding DNA and intergenic DNA. Intriguingly, around 50% of the genes coding for microRNAs have been found to be controlled by DNA methylation, and in tumor cells aberrant hyper- as well as hypomethylation of microRNA genes is detectable [43]. MicroRNAs are small non-coding RNAs that modulate gene expression by degradation of target mRNA or by inhibition of protein translation. Expression of several microRNAs has been described to be altered in RASFs and to influence proliferation rates and production of pro-inflammatory cytokines and MMPs. Levels of miR-155 and miR-146a are constitutively elevated in cultured RASFs, as well as in RA synovial tissues, synovial fluid and PBMCs, whereas the expression of miR-124a is decreased in RASFs compared to OASFs [44-48]. Expression of miR-203 is
constitutively upregulated in RASFs, and overexpression of miR-203 promotes production of IL-6 and MMPs in these cells [49]. Demethylation of normal synovial fibroblasts with 5-azacytidine led to increased expression of miR-203, strongly suggesting that global hypomethylation in RASFs might be responsible for the upregulation of miR-203. As summarized in Figure 1, epigenetic changes can already potentially explain some of the key features of the imprinted phenotype of RASFs. However, more detailed analysis of epigenetic changes in RASFs is needed to enlighten direct connections of epigenetic changes and RASF behaviour and the stage of disease development when these epigenetic changes are induced.

3.4 Do RASFs have an ‘inflammatory memory’?

Constant activation of RASFs by inflammatory cytokines or danger signals may lead to modulation of signalling pathways via epigenetic mechanisms. This imprinting of a pro-inflammatory state might not only aggravate the inflammatory state but also prevent resolution. For some signalling pathways with a known role in RA, modulating properties of the epigenetic machinery have already been described. NF-κB is recognized to play a major role in the regulation of inflammation and immune responses. The NF-κB family comprises five family members, namely p105-p50 (NF-κB1), p100-p52 (NF-κB2), p65 (RelA), RelB and c-Rel which can form different homo- and heterodimers. Upon cellular activation, inhibitor of κB (IκB), which is bound to the nuclear localization sequence of NF-κB, gets phosphorylated by IκB kinases (IKK) leading to IκB degradation. NF-κB then translocates to the nucleus and binds to consensus sequences in promoters of its target genes. HDACs and HATs are known to play multiple roles in the regulation of NF-κB activity. With its HAT activity, CBP/p300 regulates the transcriptional activity of NF-κB. P300/CBP increases NF-kB transcriptional activation via acetylation of histone tails and recruitment of RNA polymerase II [50]. Accordingly HDAC3 represses NF-kB activity [51]. Also non-histone acetylation influences signalling by NF-κB. Acetylation of distinct NF-kB p65 lysines
promotes nuclear export of NF-κB by increasing its binding to IκB, or increases NF-κB DNA binding activity [51-53]. These examples of non-histone acetylation of p65 by p300/CBP underline the complexity of transcriptional control by acetylation. Interestingly, it was found that enhanced accessibility of NF-κB consensus sequences is also mediated by the MAPK p38 pathway via induction of phosphorylation and phosphoacetylation of histone 3 [54]. This mechanism seems to be operative only in some NF-κB inducible genes, e.g. IL-8, but not in others like TNF-α, thereby enhancing NF-κB activated transcription selectively at specific loci.

It seems that epigenetic mechanisms can not only directly influence gene expression, but in parallel modulate signalling pathways leading to perpetuation of a state of increased activity. In RASFs changes in gene expression and signalling pathways induced by activating signals might be imprinted after long cellular exposure to inflammatory stimuli, become independent of the activating stimulus, and persist even in its absence. Accordingly RASFs not only react to changes in the joint environment but also actively maintain inflammation and destruction independently of an inflammatory environment and thus are co-responsible for the persistent and destructive arthritis characterizing RA.

4. Conclusion and summary

Current knowledge of the etiopathogenesis of RA suggests that the development of RA is a multistep process whereby levels of pro-inflammatory cytokines and auto-antibodies are elevated years before onset, but additional triggers seem to be needed to finally initiate disease symptoms [1, 2]. Furthermore, the fact that even though current treatments quite successfully suppress disease activity, RA cannot be cured and symptoms will flare up after discontinuation of immune suppression, points to a permanently imprinted deregulation of involved cells. For a long time it has been recognized that RASFs differ in their phenotype from normal SFs and that their destructive behaviour has become imprinted and partly
independent of activating agents. The more we discover about epigenetic mechanisms the more plausible it becomes that in RASFs epigenetic changes might be responsible for the maintenance of the activated state of these cells. In combination with a specific genetic background that predisposes for a pathological immune response and environmental factors, the pathological preservation of synovial fibroblast activation by epigenetics might be the intrinsic local component that directs the inflammatory process towards the joints. Therefore, in addition to further elucidation of epigenetically altered pathways in RA, one of the main tasks for future research will be to find the cause for this destructive memorization of an inflammatory state.

The concept that in predisposed individuals epigenetic changes lead to a pathological phenotype of stromal cells can be broadened to a variety of other chronic diseases. Among others a role for epigenetic changes has been suggested in the pathogenesis of Type 2 diabetes, chronic obstructive lung disease, chronic inflammatory bowel disease and atherosclerosis [55-58]. Complex gene-environment interactions might alter immune function and inflammatory response via changes in the epigenome and thereby promote the development of chronic diseases.

Take-home messages:

- Synovial fibroblasts are key effectors cells in the pathogenesis of RA since they produce a wide variety of mediators of inflammation and joint destruction.
- RASFs have an imprinted, activated phenotype that is independent of further activation by immune cells.
- Epigenetic changes have been found to lead to changes in gene expression in RASFs and to shape the pathogenic phenotype of these cells.
References:


Figure legend:

Figure 1. Activation and epigenetic changes in RASFs. Constant activation of synovial fibroblasts by cytokines, pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) via Toll-like or cytokine receptors might lead to modifications of transcription factors (e.g. MAPK, NF-kB), histone-deacetylases (e.g. HDAC4), histones or DNA and thereby the basal expression pattern of synovial fibroblasts in rheumatoid arthritis might be constitutively altered. Ph = phosphorylation; Ac = acetylation; Su = sumoylation; Me = methylation; TF = transcription factor.