http://pmjournal.ir Review Article

Winter 2022, Volume 7, Issue 24 (1-7)

The Role of DNA Methylation in Development and Progression of Rheumatoid Arthritis

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DOI: 10.22034/pmj.2022.252438

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Submitted: 2021-12-10
Accepted: 2022-02-15

Keywords:
Rheumatoid Arthritis
DNA methylation
Epigenetics
Treatment

Abstract:

Rheumatoid arthritis (RA) is a chronic autoimmune disease of unknown etiology that results in progressive joint destruction and ultimately to disability. Currently effective biologic therapies, exist for approximately 40% of patients, but disease activity remains inadequately controlled in others. Therefore, it is crucial to identify specific markers that predict therapeutic response in various patients, prior to the initiation of therapy. DNA methylation , as a epigenetic factor, is increasingly being explored as a potential theranostic biomarker. It has been suggested that DNA methylation might contribute to RA development, nonetheless , with conflicting results. Epigenetic modules have provided a possible interface through which genetic and environmental risk factors contribute to the susceptibility and pathogenesis of RA. Hence, epigenetic regulators may provide promising drug targets to develop novel therapeutic drugs for tailored treatment of RA patients. Here we review the current knowledge regarding the role of DNA methylation in RA and indicate its potential therapeutic implications.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial hyperplasia and joint destruction (1). Its onset is progressive and invasive, which can lead to joint deformity and disability. The disease can cause a wide variety of symptoms, clinical forms and prognoses in patients(2). While Its incidence begins to increase at the age of 25 years; it reaches a plateau at the age of 55. Furthermore, its prevalence is more than six times greater in women with 60 to 64 year-old than those in 18 to 29-year-old (3). In the industrialized world, RA affect more individuals than other countries. One third of people of all ages are also may affected by the disease at some point of their lifetime (4). The production of autoantibodies such as rheumatoid factor, and anticitrullinated protein antibodies (ACPA) are considered the hallmark of the disease that in turn supports an autoimmune etiology whereby an immune response is directed against an, as yet, unknown autoantigen. Although autoantibodies are an important characteristic of RA autoimmunity, some affected individuals may lack these autoantibodies in their blood (5, 6). The disease is complex and involves many environmental factors that trigger disease in genetically susceptible individuals (7).

Major advancements in understanding the development of RA originate from studies investigating the expression and regulation of pro-inflammatory cytokines within the affected synovial tissue. Of these pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, is a pivotal factor in the inflammatory cascade which led to its identification as a target for therapeutic intervention (8). TNF increases inflammation and tissue damage mediated by T cells, B cells, fibroblasts and macrophages in affected joints and also has systemic effects that can lead to comorbidities such as cardiovascular disease (9). Other cytokines such as IL-1 and IL-6 have similar roles in promoting the activation of T cells, B cells and osteoclasts while IL-17 promotes the infiltration of T cells and recruitment of monocytes and neutrophils, which also contribute to synovitis (10). B-lymphocytes are involved in the production of autoantibodies such as rheumatoid factor (RF) and antibodies against cyclic citrullinated peptide (anti-CCP). Differences in expression of anti-CCP and RF, rate of disease manifestation and variability of response to therapy cause heterogeneity of RA patients indicating, variation in pathophysiological

mechanisms, implication in the disease development and progression (11, 12).

In recent years, numerous studies have started to focus on the role of epigenetics in RA and investigate its contribution to the heterogeneity of patients (13). DNA methylation and histone modifications, are important epigenetic factors that affect the expression of immune-related genes and inflammation progression have become promising mechanisms to explain the pathogenesis of RA (14). Epigenetic changes in RA have been studied both in mononuclear cells of peripheral blood as well as other type of immune cells such as monocytes, T-cells and B-cells (15). In addition the epigenetic modifications in the RA synovial fibroblasts (RASFs) are of particular interest because of their aggressive phenotype, which remain stable for several passages in cell culture (16). RASFs are clue cells of joint damage and inflammation development due to pro-inflammatory and catabolic molecules synthesis, promoting abnormal proliferation and invasiveness (17). Numerous studies have found that methylation in immune cells may lead to RA progression through coordinated control of immune cell differentiation and function (18). In this review we aim to collate the current knowledge on DNA methylation in autoimmunity with a particular focus on RA, its role in altering gene expression in different cells that contribute to the pathogenesis of RA, and discuss its therapeutic and diagnostic potential.

Molecular Mechanism of RA

RA primarily affects synovial joints, in which the balance between recognition of pathogens and avoidance of self-attack is impaired and the immune system attacks and destroys healthy tissue (19). Additionally, there is increased recruitment and migration of immune cells from the bloodstream into the target tissue, including synovial membrane or synovial fluid (3, 20). Consequently, such an influx of activated immune cells producing an enhanced level of pro-inflammatory cytokines that leads to the progressive erosion of articular cartilage (21). Leukocytes, including T cells, B cells and phagocytes, are the main types of immune cells in the rheumatoid synovium (22). In fact, macrophages and granulocytes produce chemokines, pro-inflammatory cytokines, and reactive oxygen species, which are associated with classical inflammation. Besides, B lymphocytes play critical roles in the pathogenesis of RA (23). They are the main source of ACPAs and RF which involve in the formation of immune complexes as well as complement activation in the joints (24). B cells associated with the pathogenesis of RA disease not only activated by the presentation of antigens but also play a pivotal role in the development of the disease due to the production of antibodies, anti-self-antibodies and cytokines. In addition to the role of soluble pro inflammatory molecules and activity markers, such as TNF, interleukin (IL)-6 and C-reactive protein, in the pathogenesis of RA, local synovial cellular interactions drive the key processes of long-term cellular proliferation and destruction of the rheumatoid joint (25, 26).

influencing factors in RA

People born with certain genes, called HLA class II genotypes, are more likely to develop RA (27). Although it is undeniable that genetic factors play the major role in the susceptibility of the illness , nonetheless, the low concordance rate (12–20%) observed in monozygotic twins suggests that environmental factors may also play a significant role in the pathogenesis of RA (28). Environmental factors such as smoking and infections may have affect the incidence of the disease as well as the rate of progression and severity of the RA (29). Moreover, other known environmental factors such as latent viral infections, sex hormones and deficiency of vitamin D may influence the disease (30). It is thought that these environmental factors influence epigenetic modifications, which in concert with the individual genetic susceptibility status result in the development of RA symptoms. Genetic heterogeneity however, does not explain all the features of illness (31, 32). Thus, investigation of epigenetic factors and mechanisms associated with the progression of the disease and response to treatment is increasingly important. Nevertheless, Investigating the epigenetic landscape can provide novel therapeutic targets (5, 33).

Treatment for arthritis rheumatoid

The main treatment goals are to control inflammation, ease pain, and reduce disability linked to RA (34). Current treatment guidelines recommend that patients initially treated with a combination of corticosteroids and disease-modifying antirheumatic drugs (DMARDs) to slow down disease progression and reduce synovitis along with disability (35). Though many people with RA need to take more than one drug to combat the disease. This is because drugs work in different ways to reduce the symptoms(36). There are three types of DMARD:

•conventional synthetic DMARDs (sometimes called csDMARDs)

•biological therapies (sometimes called bDMARDs).

•targeted synthetic DMARDs (sometimes called tsDMARDS)

Some DMARDs include hydroxychloroquine (Plaquenil), leflunomide (Arava), sulfasalazine (Azulfidine), or tofacitinib (Xeljanz) (37, 38). Although steroids are sometimes known by their full name: corticosteroids, it helps to reduce the pain, stiffness and inflammation caused by RA (39). The most common anti-inflammatory steroids

include hydrocortisone (Cortef), methylprednisolone (Medrol), and prednisone (Deltasone) (40). Non-steroidal anti-inflammatory drugs (NSAIDs) can be used to help control symptoms of pain, swelling or stiffness. They commonly used in combination with painkillers (41).

Biologic therapies are genetically engineered human proteins that specifically target inflammatory cytokines, such as TNF-α and IL-6 or immune pathways, such as CTLA-4 costimulatory pathways, or B cells (42). Though when bone damage from the arthritis become severe or pain is not controlled with medications, surgery is an option to restore function to a damaged joint (43).

Mechanism of DNA methylation

Epigenetics refers to chemical modifications that influence gene regulation without changing the DNA sequence (44). These alterations include DNA methylation and post-translational modifications of histone proteins(45). This review will focus mainly on studies of DNA methylation as a biomarker of response to treatment in RA. DNA methylation is a heritable epigenetic marker involving the covalent transfer of a methyl group to the C-5 position of the cytosine ring of DNA by DNA methyltransferases (Fig1) (4, 46). However, more than 98% of DNA methylation occurs in a CpG dinucleotide context in somatic cells, while as much as a quarter of all methylation appears in a non-CpG context in embryonic stem cells (ESCs) (47).

DNA methylation is a more stable biomarker than gene expression, and aberrant methylation has been reported in several cancers (48). DNA methylation has also been found to predict response to therapy; for example, an epigenome-wide association study (EWAS) identified methylation signatures as a predictive response to anti-EGFR, a common therapeutic for metastatic colorectal cancers (49, 50).

Impaired DNA methylation in RA

Whole genome analysis of aged individuals has highlighted a number of hypomethylated regions that may contribute to age-related diseases, including some autoimmune diseases such as RA (51). The first evidence to suggest that DNA methylation may play a role in aging and autoimmunity came from studies investigating the effect of the DNA methyltransferase inhibitor 5-azacytidine that can induce symptoms associated with autoimmunity (52). There is emerging evidence of the interrelationship between DNA methylation and inflammation in regulating immune pathways. For example, the cytokine, IL-6, has been reported to increase the expression of DNMT1, levels of which correlate with DNA methylation in T cells (53). Studies have shown that the extent of methylation regulates migration, differentiation, and activation of T-cells. T cell activation leads to demethylation of the interleukin-2 promoter, resulting in interleukin-2 production (54). Such impairment of DNA methylation may play a role in RA pathogenesis. Specifically for RA, differential DNA methylation has been demonstrated in the IL-6 promoter (55). In 2008, Nile and colleagues found that IL-6 promoter methylation reduced transcriptional activity and identified a single CpG within the IL-6 promoter that was key to regulating IL-6 gene expression (53).

Recent studies confirmed a global DNA hypomethylation in T-cells and monocytes of RA patients compared to healthy individuals (56). In CD4+ T cells, 383 hyper- and 785 hypo-methylated genes were identified in RA patients ($p < 3.4 \times 10\text{-}7$), including three regions within HLA that were frequently

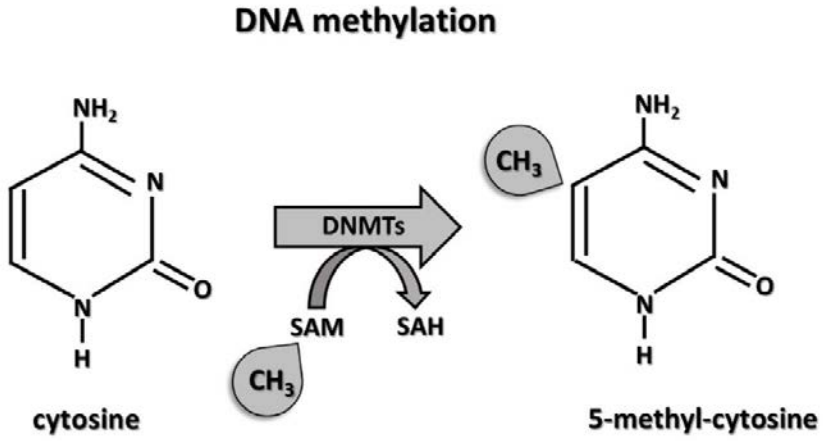

Fig1. DNA methyltransferases (DNMTs), responsible for the transfer of a methyl group from the universal methyl donor, S-adenosyl-L-methionine (SAM), to the 5-position of cytosine residues in DNA (46).

hypomethylated (57). Genome-wide analysis of DNA methylation by microarrays revealed its impact in B-cells on the early stages of patients who have not yet received treatment compared to healthy donors. Genome-wide methylation change was also found in T and B (58). A study identified 150 and 113 CpG loci with unique methylation characteristics in T and B lymphocytes in patients with ERA.

Rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) are involved in the release of inflammatory mediators and matrix degrading enzymes, which are key effector cells leading to synovial inflammation and destruction of bone and cartilage (59). The changes in DNA methylation in RA-FLS play important roles in the pathogenesis of illness. Hypomethylation in RA-FLS may be caused by the downregulation of DNMT1 and DNMT3A after inflammatory environmental stimulation (60).

DNA methylation as a biomarker in RA

Biologic drug therapies represent a huge advance in the treatment of RA (61). However, effective treatment of disease trol is achieved in only 30% of patients, making identification of biomarkers of response a research priority. Since DNA methylation appears to have a role in RA pathogenesis, it may also be a suitable biomarker of treatment response (62). Just as serologic and genetic studies indicate that there may be more than one sub-type of RA with a wide range of responses to biologic treatment, differences in baseline methylation status may also serve as a marker of varied disease subtypes that might respond better to therapies targeting the particular pathway involved (63). DNA methylation may thus provide a biomarker of subsequent treatment response (64). It is urgent to find novel markers to augment the diagnostic accuracy, prediction of disease onset , and its progression. For example Methylation levels of SHROOM1 in ERA are substantially increased, hence it can be applied as an early diagnostic biomarker (65). Additionally, identification of aberrant DNA methylation may change disease onset which in turn it might lead to a better understanding of the risk factors that contribute to disease development and thus result in the identification of specific biomarkers for disease analysis(66). Recently, reverse transcriptase (RT)-PCR assays have been developed to quantify the number of Foxp3+ cells within RA tissue samples (67).

It should be noted that there may be other appropriate biomarkers of response. Micro-RNAs (miRNA) are small, noncoding RNA structures that act as regulators of gene expression (68). There is increasing evidence that implicates dysregulation of miRNA in blood, T cells, and synovial fibroblasts in inflammation and joint destruction are found in RA patients. There have been a number of successful therapies for patients, however , a proportion of patients fail to respond to conventional therapy (69). Ideally, it would be useful to identify this fraction of nonresponders earlier in the course of the disease, to provide better treatment regimes that are tailored towards the individual patient. It is becoming clear that RA patients display a differentially methylated genome when compared to healthy individuals (70). This raises the possibility that measuring DNA methylation patterns of responders and nonresponders may lead to the use of DNA methylation as a predictive biomarker for treatment response (71).

CONCLUSIONS

In recent decades, many studies have shown that epigenetic mechanisms are involved in the regulation of all biological processes in the body from impregnation to death. In recent years, a major advancement has taken place in understanding the role of DNA methylation in the pathogenesis of RA, hence it can be used as effective biomarker in the disease process (72). It is hoped that the progress made in identifying epigenetic mechanisms occurring in cancer can also be exploited in inflammatory disease for other disease assessment. Unfortunately, studies on the clinical use of epigenetic drugs modulating aberrant DNA methylation patterns in RA are at a very early stage. More research should be conducted on DNA methylation in regard to treatment and diagnosis of cancer and proliferative diseases (73). Moreover, identified differential methylation genes can be applied as useful biomarkers to predict disease progression and severity and also provide potential therapeutic targets for RA. Epigenetic modifications as drug targets could provide a new direction of pharmacological research for the development of novel drugs that alleviate symptoms of high toxicity, low efficiency, and high cost of the current medical care. For example, demethylation of FOXP3 is used as a biomarker to evaluate the therapeutic drug response, which provides a direction for the precision treatment of RA (74). It is crucial to find new DNA methylation biomarkers that can be used in everyday practice to detect early onset of RA before the induction of irreversible joint destruction . These knowledges collectively may not only delay or reduce disease progression but also it decreases the costs of health care (75).

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