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Impact of Epigenetic Regulation on the Pathogenesis and Inheritance of Rheumatoid Arthritis

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Abstract

Rheumatoid Arthritis (RA) is an autoimmune disease that causes joint and bone damage through inflammation. This chronic condition is often linked to genetic and environmental factors. With advancements in the field of epigenetics, increased speculation has arisen to understand the impact of DNA methylation, microRNAs, and histone modifications on the progression of RA. Studies have indicated that dysregulation in epigenetics significantly contributes to the pro-inflammatory landscape of RA. Furthermore, separate lines of evidence have shown that altered epigenetic patterns due to the disease can be inherited. This literature review aims to compile all available evidence regarding how epigenetic mechanisms contribute to the pathogenesis and inheritance of RA.

Keywords: rheumatoid arthritis, epigenetics, inheritance, DNA methylation, histone modification, MicroRNAs, transgenerational, acetylation

1. Introduction`

1.1 Introduction to Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a chronic inflammatory, systemic autoimmune disease that

affects the synovial joints of the body, caused primarily by the combination of genetics and environmental factors. The immune system is the body's defence regulator that maintains tissue and cell health by fighting bacteria, viruses and infections that could cause irregularities in bodily functioning. Additionally, it is responsible for aiding disease prevention, destroying abnormal cells, storing information in the form of memory about pathogens, reducing tissue inflammation and working to repair cellular damage (Chaplin, 2010). However, autoimmune diseases refer specifically to diseases caused by the dysfunction of the immune system, leading to its inability to distinguish between healthy cells of the body and foreign pathogens that can cause cellular damage. The "self-attack" pathway involved in immune response often causes inflammation and leads to physiological and structural damage of tissues and organs.

The progression of this disease leads to joint destruction, cartilage loss and bone erosion, due to which it requires treatment. The review by Fugger L. et al., 2000 used mouse and human models to detail the association between RA and Class 2 Major Histocompatibility complex (MHCs). Class 2 MHC antigens are the surface proteins used by the immune system to detect the presence of foreign pathogens. These antigens are presented to Helper T cells upon detection, triggering cytokine release as a response. In this way, MHC alleles such as HLA-DR4 are responsible for producing pro-inflammatory cytokines that cause joint damage and tissue inflammation. Among the cytokines, TNF-**α** in the synovial joints has been the most well-studied. Scientists have postulated that HLA-DR4 and inflammation-related genes are the cause of RA. However, there have been several discovered causes beyond genetics, too. There is evidence for environmental effects and epigenetics, too. This review will focus on the epigenetic mechanisms involved in the development of RA and its contribution to RA inheritance.

A review by Silman et al., 2002 statistically analysed that around 1% of the population has RA, with numbers being almost double in the female gender. While there exist populations with higher susceptibility rates to this disease, such as the Pima Indians with over 5%, there also exist certain populations with an extremely low number of occurrences, such as in China and Japan. Hence, we see RA as a condition that can be genetically inherited. The alleles of TNF, the inflammatory cytokines, also impact the progression of RA. However, many lines of evidence discussed in the paper suggest that environmental factors are equally important. Lifestyle choices and habits significantly impact hormone levels especially sex hormones in women, which regularly fluctuate during ovulation, pregnancy and menopause. While pregnancy in itself reduces the risk of RA, continuous drastic fluctuations of hormone levels are linked with an increased risk of RA. (Sokka, T. et al, 2009)

Due to its self-induced and complex nature, most autoimmune diseases including RA can't be cured, and instead must be kept in control to reduce chances of comorbidities. Thus, autoimmune diseases have come to become an increasing financial and physical burden on the lives of patients, whose quality of living is compromised. Chronic inflammation specifically, can lead to cellular and tissue damage that can impair organ systems. Inflammation has high cardiovascular and neurological risks as well, such as heart attack, strokes, and cancer development.

1.2 Inflammation in Rheumatoid Arthritis

The immune system refers to the cells, tissues, and organs that work cohesively as the body's defense against foreign pathogens and infections. The immune system is responsible for detecting substances that are detrimental to bodily function and destroy them. Inflammation is one such critical immune response against infectious pathogens. Numerous proteins and hormones in the body have been found to promote chronic inflammation, including proinflammatory cytokines, interleukins, growth factors, and chemokines. The review (Kany et al., 2019) about cytokines and their roles in inflammatory diseases explains that cytokines are secreted proteins that regulate (promote/inhibit) immune responses. Interleukin (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) are the proinflammatory cytokines primarily studied in RA due to their role in tissue and cell damage. The simultaneous release of IL-6 and TNF-α induces an anti-inflammatory response to maintain homeostasis, however these aren't present in the case of RA.

The cell populations most studied in RA are T cells, B cells and lymphocytes. While B cells are most linked with their role in producing antibodies, T cells can be either cytotoxic or regulatory. If T Cells express CD4, it is converted into a T-Helper Cell (Th). These T-helper cells are further specialized and involved in producing cytokines and antibodies by stimulating B cells to destroy foreign substances. (Cano, R. et al, 2013). The common phenotypes of T-Helper cells are Th1, Th2, Th9, Th17, Th22, and T-reg, each known to produce a different cytokine.

Overall, autoimmunity and inflammation leads to bone destruction and deterioration in RA.

1.3 Epigenetics

Epigenetics refers to post-transcriptional modifications that change gene expression without impacting the DNA sequence. The genome can be regulated by activating or silencing the gene whose region the modification is made in. Common post-translational modifications include DNA Methylation, miRNAs and histone modifications such as acetylation, phosphorylation and ubiquitination. By activating/silencing genes, epigenetics causes developmental changes, genetic diversity and allows cellular differentiation. (Handy et al., 2011)

DNA methylation is an epigenetic modification that regulates gene expression in the mammalian genome. It includes the transfer of a methyl group to the C-5 position of nitrogenous bases to create for example, 5-methylcytosine. This reaction in specific, is catalyzed by a category of DNA methyltransferases (DNMTS) that carry out methylation by transferring a methyl to Carbon 5 of a cytosine from a S-adenyl methionine. (Moore et al., 2013) DNA methylation impacts gene expression by modifying the types of interactions that occur between DNA, chromatin proteins and transcription factors. DNA methylation can occur at different loci and regulate different genes cause phenotypic alterations. While DNA methylation is commonly associated with gene inactivation, transcription factors and regulatory proteins can be bound to DNA or work to both activate and silence it. DNA methylation of genes related to the pathogenesis of RA has been documented and will be reviewed further in the results/of this study.

MicroRNAs, commonly known as miRNAs are smaller, non-coding strands of an RNA molecule that typically range in length between 18-25 nucleotides long. They act as an extra layer of regulation on post-translational gene expression, being vial in cell differentiation, host-pathogen interactions, and tumorigenesis. MiRNAs undergo a series of pathways to convert them into mature strands, which when loaded into the RISC (RNA-induced silencing complex) can then lead to translational repression. The maturation of miRNAs is facilitated by two processing enzymes, Dicer and Drosha, which allows the mature product to bind to cellular transcripts (Hammond, 2015). Dicer and Drosha work to process RNA into microRNA by cleaving the dsRNAs into smaller pieces (Kuehbacher et al., 2007). There is miRNA regulation present in inflammation-related genes. If these miRNAs are dysregulated, as seen in the case of RA, it can skew inflammation.

Histone modifications refer to those post-translational modifications of histone proteins, which make up the core of the nucleosome that assists the supercoiling of the DNA. The presence of histone modifications in different permutations become characteristic of the assembly/disassembly of the nucleosome (Okuwaki M, et al, 2010.). Histone modifications are most found to be involved in the covalent binding of different chemical groups to one of the four core histone proteins (H2A, H2B, H3, H4). The common binding chemical groups are acetyl, methyl, and phosphate. Histone acetylation is carried out by histone acetyltransferases, proteins that transfer an acetyl group from Acetyl CoA (cofactor) to the ε-amino group of lysine side chains. The transfer neutralizes the positive lysine charge, resultingly weakening the supercoiling interactions between DNA and histone proteins (Sun X, et al 2015). In phosphorylation, histone kinases transfer a phosphate group from ATP onto a hydroxyl (-OH) group on the side chain of bases, making them negative (Bannister & Kouzarides, 2011). The change in charge impacts chromatin structure and binding. Methylation, which occurs primarily on lysine and arginine side chains, does not change the charge around the histone.

Beyond structure and regulation, histone modifications also impact genome stability and facilitate damage repair in the DNA strand. Environmental factors also contribute to histone modifications, and alter the pathways and function of the enzymes that are responsible in carrying out these processes. These factors include oxidative stress in highly polluted regions, diet-induced enzyme modifications among others; these can begin altering the genome as early as in the pre-natal stages. (Dai & Wang, 2014). Such histone modifications, especially methylation and acetylation, have been studied and found to have a strong effect on the disease development and progression of RA which will be discussed in this paper.

1.4 Relationship between Epigenetics and Rheumatoid Arthritis

Earlier studies that studied the etiology of Rheumatoid Arthritis (Liao et al., 2009) concluded its results to be driven by both genetic predispositions and environmental factors. Twin studies performed to understand the genetic association showed that the heritability of RA was over 60% (Yarwood et al., 2016). The study also showed that first-degree relatives of RA patients were most susceptible to also being diagnosed with RA in the future (Sparks and Costenbader, 2014). A more pathological view shows that RA occurs due to autoimmune

inflammation in the synovial membranes of the joint. Cells respond as they would in the presence of a tumor, causing bone deterioration and cartilage erosion.

Tissue dysfunction of the synovial membrane increases its permeability, allowing penetration by macrophages, fibroblasts and many active lymphocyte-producing cytokines such as T-lymphocytes and B-lymphocytes. The production of cytokines such as Tumor Necrosis Factor (TNF), Interleukin (IL) and autoantibodies like Rheumatoid Factor (RF) and anti-CCP can moderate the progression of RA and dictate the variability of response to different types of therapies (Nemtsova et al., 2019). Gene expression can be epigenetically controlled and is, therefore, a necessary area of study to learn more about RA's causes and potential therapies.

Recent studies exploring the role of epigenetics in RA disease progression most commonly study the mechanism of DNA methylation due to the advanced technology and available preexisting knowledge (Chen, J and Yan, Q, 2017). While earlier methylation studies found variability in the methylation changes of specific genes such as Interleukin-6, Interleukin-10 and CXCL12, newer Epigenome-Wide Association Studies (EWAS) have been able to identify specific DNA methylation changes on specific genes that can be identified as biomarkers of RA. However, more research needs to be conducted regarding histone modifications and microRNAs' role in regulating the progression of RA.

In this paper, we hope to gain some more insight into how the relevance of genetics in the development of Rheumatoid Arthritis is linked with inheritance as well. As seen, alleles of Tumor Necrosis Factor and HLA-DR4 change the plausibility of developing the disease, which, when inherited by offspring, could significantly impact their lives. However, the paper also aims to consider the environmental factors causing changes at this genetic level and whether these can be carried on transgenerationally.

2. Method

This literature review sought to explore the interplay between transgenerational aspects, DNA methylation, histone modification, microRNA, and their relevance to Rheumatoid Arthritis (RA). The research methodology encompassed a comprehensive online search for primary and secondary papers conducted on PubMed (NCBI) and Google Scholar. To identify relevant literature, a variety of specific terms were employed, including "Transgenerational," "Rheumatoid Arthritis," "DNA Methylation," "Histone Modification," "microRNA," among others, utilized in varying combinations to maximize information retrieval.

The timeframe for the literature search spanned from June to August 2023, while the selected timeline for extracting articles encompassed publications from the year 2000 onwards. This time frame was selected deliberately, recognizing the relatively recent advancements in the field of genetics, thereby capturing the most contemporary and pertinent research.

The inclusion criteria for the papers considered encompassed studies involving both animal and human subjects related to the specified topic. This approach ensured a comprehensive understanding of the intergenerational aspects and genetic mechanisms associated with RA.

The process of literature acquisition involved scrutiny and selection to incorporate studies

that delved into the molecular aspects and hereditary implications of RA. The inclusion of animal and human studies facilitated a comprehensive analysis of the transgenerational elements associated with DNA methylation, histone modification, and microRNA in the context of Rheumatoid Arthritis.

3. Results

3.1 DNA Methylation in RA:

3.1.1 Overexpression of Pro-inflammatory Cytokines

In the human body, Interleukin-6 (IL6) and Tumor Necrosis Factor-α (TNF) are the proinflammatory cytokines primarily studied due to their role as regulators. Regarding tissue damage, the two cytokines via immune activation cause chronic inflammation, tissue damage, and cardiovascular and gastrointestinal issues. The pathology in RA comes from an upregulation of proinflammatory cytokines, leading to tissue damage and the joint disintegration seen in the disease. Hence, recent studies (Geginat et al., 2016) have noted the importance of a simultaneous release of pro and anti-inflammatory cytokines to ensure an appropriate immune response to maintain immune homeostasis. However, in chronic diseases such as RA, there is evidence for much higher levels of proinflammatory cytokines promoting inflammation (Brennan F, 2008)

IL-6 and TNF-α blocking treatments and therapies are successful in reducing the impacts of Rheumatoid Arthritis due to their role in promoting inflammation. A recent study by (Mao S-Q et al. 2017) explored the role of DNA methylation in inflammation. It was based on the idea that inflammation and endothelial dysfunction are the primary causes of elevated blood pressure levels. The study investigated DNA methylation of IL-6 in an inflammatory state, and the results revealed that the IL6 promoter was hypomethylated, which increased gene expression. Increased IL6 levels are a characteristic cytokine indicator in the pathogenesis of RA. The study also studied how environmental factors such as gender, smoking and health controls impacted the same.

Another study (Karouzakis E et al. 2009) focused on the methylation patterns of Rheumatoid arthritis synovial fibroblasts (RASF), which are activated fibroblasts that contribute to RA inflammation, bone decay, and damage. The study revealed that RASF DNA had lesser 5-methylcytosine and methylated CG sites upstream of the reading frame. Additionally, reproducing the activated phenotype of the RASFs in normal Synovial Fibroblasts using 5-azaC showed that the up-regulation increased by over double due to hypomethylation. RASFs produce matrix-degrading enzymes that decrease support matrices in joints. The study by Karouzakis further showed that in RASFs, there was hypomethylation in the promoter region of matrix-degrading enzymes, causing upregulation. This upregulation contributed to joint destruction in RA.

3.1.2 Immune Cell Dysfunction

Regulatory T cells limit the impact of chronic inflammatory diseases such as RA and maintain peripheral tolerance (Viganli, D. et al, 2009). CTLA-4, or cytotoxic

T-lymphocyte-associated antigen 4, is a “negative regulator of T-cell function.” When active, it promotes the inhibition of immune responses, especially in tumours, and when inhibited, it results in the immune system being activated. A study by Cribbs et al. conducted in 2014, in an attempt to understand why in Rheumatoid Arthritis T-reg cells had impaired function and low levels of CTLA-4, concluded this to be due to DNA methylation patterns (Cribbs, A. et al, 2014) The study measured the CTLA-4 expression in RA patients which’s results explained that the down-regulation of CTLA-4 was due to DNA methylation at a binding site of the CTLA-4 gene promoter region. Due to the methylated CTLA region, gene expression of pathways that control inflammation, such as the kynurenine pathway, is prevented. This pathway is dedicated to controlling inflammation and long-term immune response, which is also the dysfunction implicated in RA progression.

Similarly, when considering the role of T-reg and Th17 as regulators of the immune response, a study conducted in 2020 by (Huang Y et al. 2020) investigated how different stages of the diseases impacted the Treg and Th17 levels and whether DNA methylation was involved in causing this, and in the development of RA. The method called for 65 patients in different stages of RA development, along with a few healthy controls. The results of a post-studying methylation pattern of genes revealed that patients with early RA had much lower levels of Treg/Th17, while the expression of IL-6 and IL-17A was relatively higher, and IL-10 and TGF-B were lower.

3.1.3 Citrullination

Citrullination refers to modifying the amino acid arginine to the amino acid citrulline post-translation. This biochemical conversion is carried out by peptidyl arginine deiminase enzymes, or PADs, which are a group of enzymes that hydrolyze arginine guanidinium to urea. The study by (Alghamdi M et al. 2019) examined how these citrullinated proteins, which are non-essential in the human body, can sometimes be recognized as nonself antigens, leading to an autoimmune response. The most commonly citrullinated proteins modified in patients are fibrinogen, collagen II, vimentin, etc. As explained, each of these proteins has specific sites the immune system recognizes as an antigen. The humoral response that results leads to the formation of autoantibodies known as anti-citrullinated peptide antibodies. These antibodies are known to cause the breakdown/destruction of these citrullinated proteins and even the surrounding tissue and cell matter, which results in different autoimmune disorders.

RA patients exhibit increased citrullinated proteins alongside immune responses, since the immune system attacks citrullinated proteins (Darrah E et al., 2019). This makes it a hallmark characteristic of RA disease progression. Once modified there is a change in the structure since there is a shift in the charge, which consequently changes the molecular interactions. In general circumstances, citrullination occurs during apoptosis or cell death; citrullinated proteins and enzymes play a role in maintaining inflammation, especially in the joints. Citrullinated proteins can be found freely floating or cell-bound and are targeted by autoantibodies to trigger an inflammatory response.

DNA methylation is the modification commonly associated with the expression of genes that code for the transcription of proteins; similarly, in this way, methylation can be seen regulating the production of PADs as these are enzymes.

A recent study (Kolarz B et al., 2020) conducted an experiment to assess whether the promoter region of the PADI4 gene, which is responsible for the production of the PADs was susceptible to epigenetic regulation. The study consisted of 155 patients, 125 of whom were patients of RA and the rest as controls. The result of the study was considerably groundbreaking as it found that the gene promoter region of PADI4 does undergo methylation. The experiment found that the hypomethylation of PADI4 in RA patients increased citrullination, consequently producing more ACPAs due to more antigen detection. Therefore, it reinforces the consequent idea that higher methylation of the PADI4 region would result in reduced production of this enzyme, and hence, we would see fewer citrullinated-modified proteins. Lesser citrullinated proteins would also be the reason for lower ACPA production, which would reduce the tissue damage caused, hence lowering the activity of RA. There is also evidence that the reason autoimmune disorders may appear more in women is because changes influence PAD enzyme activity in hormone levels. (Venrooji W, et al 2000)

3.2 microRNAs in RA:

3.2.1 Angiogenesis

Angiogenesis refers to the formation of new blood vessels from existing venules. Angiogenesis is crucial in development as it facilitates wound healing and inflammation, critical immune responses. Any imbalances in angiogenesis directly impact RA since the newly developed blood vessels are responsible for nutrient and oxygen transport to the site of inflammation, which is how the proliferation of synovial tissue occurs. However, angiogenesis-stimulating factors and angiogenesis inhibitors (Lugano, R. et al, 2020) control the regulation of angiogenesis, which either promote or inhibit the process of new vessel production. Increased angiogenesis is often seen alongside inflammation, so angiogenic factors are commonly studied as indicators of disease progression in RA. The review by (Elshabrawy H. et al. 2015) explains how angiogenesis is triggered when pro-angiogenic factors dominate the endogenous angiostatic mediators. The synovial tissues release these factors. Pro-angiogenetic factors that contribute to RA are not limited to such growth factors but also include cytokines and chemokines.

A study released in 2006 by (Poliseno, L. et al. 2006.) attempted to assess the impact of epigenetic regulation on angiogenesis. It attempted to research how post-transcriptional modifications, specifically microRNAs, impact in vitro angiogenesis. This was done by analysing microRNA expression in Human Umbilical Vein Endothelial Cells (HUVEC); of all the miRNAs analysed, 15 contained angiogenic factor receptors, providing evidence of their involvement in the process. Specifically, miR-221 and miR-222 affect the gene c-Kit, crucial for mast development and cell survival. The interaction between miR-222 and c-Kit was shown to be responsible for epithelial cell control involved in new vessel development. miR-222 is commonly related to the regulation of gene expression, whereas c-Kit has a more direct impact as it regulates the activation of synovial fibroblasts.

Newer studies (Sun L. et al., 2018) have discovered that miRNAs are further involved in angiogenesis regulation, including its promotion and regulation. For instance, miR-155 has been found to target genes and promote vascular endothelial growth factor (VEGF) signalling.

This microRNA is commonly seen upregulated in the synovial tissue of RA patients, explaining that it contributes to promoting angiogenesis and RA pathogenesis. Contrastingly, miRNAs such as miR-16 have been found to be inhibitory of angiogenesis and shown to target pro-angiogenic factors. This conclusion was confirmed due to finding it downregulated in the synovium of RA patients, explaining that due to its role as an inhibitory gene-modification it would be higher in non-RA patients.

Furthermore, angiogenesis regulation is not only limited to miRNA but is also seen being impacted by Dicer and Drosha, the two enzymes responsible for miRNA splicing and formation. Animal studies conducted in the past (De Cauwer, A. et al., 2018.) assessed this by splicing the Dicer gene from the exon of the first two amino acid sequences to make the gene defective. The results showed that the mice could not develop vasculature properly with these defective DICER enzymes. Hence, in RA, we see modifications by miRNAs on genes involved with the process of angiogenesis. Based on their regulatory patterns, these genes can either promote RA pathogenesis and inflammation by producing growth factors or limit inflammation by down-regulation genes coding for inflammatory proteins. (Tiwari, A. et al 2017)

3.2.2 Bone Destruction and Deterioration

Bone destruction and deterioration are some of the most common consequences of Rheumatoid Arthritis. This destruction is the precursor for joint dysfunction as well. Patients at an early stage of the disease often face this in the form of bone loss or bone erosion, which worsens with time (Schett et al.; E. et al., 2012)]. However, the main reason for bone damage in RA is an imbalance in bone metabolism, which occurs when osteoclast activity is either increased or decreased beyond the equilibrium. Osteoclasts are essential in bone development due to their mediation in the process of bone resorption and bone formation, and any deviation from this can act as a biomarker for RA. (Yap, H. et al, 2018.)

The regulation of osteoclasts, which impacts their activation/inhibition, is found to be mediated by the factors which exist in the synovium. Studies such as (Yao, Z. et al., 2021.) have found that pro-inflammatory cytokines such as TNF-a and IL-1 play an important role in osteoclast differentiation. In Vitro, studies also provide evidence of the involvement of IL-1 in both cartilage and bone destruction by promoting the production of matrix proteins capable of degrading cartilage and bone tissue. (Abramson, S. et al., 2002). While TNF-α specifically contributes by promoting osteoclast differentiation at an early stage. Osteoclasts are extremely important in RA, as when assessed in a study, any animals with inflammation that could not produce osteoclasts did now show evidence of bone resorption. Hence, osteoclasts are seen impacting bone erosions and regulating the degree of bone loss in RA patients.

However, epigenetic evidence shows how miRNAs contribute to the regulation of osteoclastogenesis and the growth, development and differentiation of osteoclasts. As per our understanding of epigenetics and its involvement in modifying genes post-transcription, such changes would explain why there is an imbalance in the levels of IL-1, TNF-alpha and other such molecules responsible for contributing to osteoclast regulation. Past research found the levels of miR-145-5p elevated in the peripheral blood mononuclear cells and synovial tissue

in RA. A following study (Wang, X. et al. 2015) aimed to explore the reason for these elevated levels for the same. An animal study conducted using a collagen-induced arthritis mouse model (Alabarse, P. et al., 2015) found that overexpressed miR-145-5p upregulated RANKL and osteoclast differentiation. After injecting the mice with the agomir of miR-145-5p and assessing the bone changes over four weeks, it was evident that the erosion in the joints had worsened. Therefore, we can understand that, as explained above, pro-inflammatory cytokines interfere with osteoclast function and deteriorate bone health, along with the general malfunction of osteoclasts in RA. The cytokines and malfunctioning osteoclasts, regulated at a microRNA level, lead to joint destruction.

Other miRNAs have also been involved in expressing proteins that control bone homeostasis. For instance, mir-146a disrupts biochemical pathways involved in regulating osteoclasts, upregulating them to cause increased bone erosion. This is done by the miRNA modifying genes coding for proteins such as interleukin-1 associated kinase 1 (IRAK1) and TRAF6. Additionally, miRNAs such as miR-124, which works to inhibit the RANKL pathway, are also commonly downregulated in the synovial tissue of RA patients, promoting RANKL and increasing bone erosion.

However as seen, there are many more miRNAs that based on their function are either upregulated or downregulated in RA. Refer to Table 1 below for additional examples of papers that have investigated the mechanism of regulation in miRNAs not discussed above.

Table 1. microRNAs implicated in the pathogenesis of inflammation in rheumatoid arthritis

miRNA	Tissue	Regulation in RA	Physiological function of miRNA	miRNA role in RA pathogenesis	Citation
miR203	Rheumatoid Arthritis Synovial Fibroblasts (RASF) isolated from patients	Upregulated	Regulates proliferation, apoptosis. Multi-system function: skin maintenance, increased tumor development, decreased tumor suppressor, increased pro-inflammatory	Increased secretion of IL6 and MMPs	Stanczyk J, et al 2011.
miR155	Synovial Tissue isolated from RA patients	Upregulated	Regulates inflammatory response of monocytes and T cells. Regulate proliferation, apoptosis, invasion and secretion of inflammatory cytokines.	Decreases secretion of FOXO3A, increases secretion of IL-1B, IL6, TNF-α	Wang, Y et al 2019.
miR22/222	Serum & Synovial tissue from RA patients	Unregulated	Regulates cell growth, oncogenesis, invasion, migration and drug resistance in tumor cells	Increases expression of TNF-α, IL-6 and IL-1β, triggers VEGF, MMP3 and MMP-9 regulation	Yang S, et al 2015
miR199	Venous blood samples from RA patients	Downregulated	Regulates a variety of cell growth behavior. Anti-proliferative,	Suppresses RB1 mRNA, which inhibits RAFLS	Wangyang Y, et al, 2018.

			Anti-invasive, Anti-inflammatory	proliferation and induces apoptosis	
miR137	Fibroblasts like synoviocytes from animal-induced rat RA models	Downregulated	Regulator of susceptibility genes in diseases such as lung, gastric, renal and breast cancer	Targets CXCL12 which is vital in angiogenesis and tissue repair	Du, J et al 2017.

3.3 Histone Modifications in RA

Histone acetylation is one of the post-translational histone modifications in which adding an acetyl group to the N-terminal of a histone can affect gene expression. It does this by chemically modifying the chromatin structure. As seen above, most of the biochemical processes in RA are carried out by proteins, enzymes, or growth factors that promote inflammation. One of the key genes involved in RA is the FOXP3 gene, which codes for the transcription factor FOXP3, which is crucial in regulating T-reg cells. T-reg cells are classified as the immune cell class that regulates immune response within tolerable bounds. Hence, any dysfunction in the number of T-regs, especially when lesser in quantity, leads to autoimmune diseases due to the imbalance. Therefore, it is essential that the FOXP3 gene is regulated, as it is responsible for maintaining this immune balance. The FOXP3 transcription factor, among others, controls inflammation by inhibiting the expression of pro-inflammatory cytokines while promoting the expression of genes related to T-reg cell production.

However, studies (Xiao, Y. et al., 2010) have now revealed that post-transcriptional histone acetylation modification is responsible for the regulation of the genes. Histone acetyltransferases (HATs) chemically modify a targeted histone protein by adding an acetyl group onto it, which impacts the chromatin structure. In RA, there is a general case of downregulation caused by decreased acetylation at the gene loci specific to the expression of Fox P3. This decreased expression of the gene results in a lesser quantity of FoxP3 transcription factors that work to maintain immune homeostasis due to their anti-inflammatory response by T-reg cells. Hence, any shift in expression patterns due to acetylation, significantly if decreased acetylation, can promote immune instability and inflammation in diseases like RA.

Similarly, histone acetylation has also been found to impact the expression of IL-6, one of RA's major pathogenic inflammatory cytokines. A study (Wada, T. et al., 2014.) found that the acetylation of the H3 histone protein was relatively much higher in RASFs than in regular Synovial Fibroblasts. This could explain why RA patients are known to have elevated IL-6 levels, as histone acetylation of a gene is usually associated with increased expression. Due to this, the IL-6 promoter region of the chromatin is loosely coiled. These elevated levels of production trigger a response of histone deacetylases, or HAT inhibitors, to respond by reducing the levels of the H3 acetylation in the IL-6 promoter region. However, HAT inhibitors such as Curcumin, further respond by reducing IL-6 levels by directly targeting IL-6 mRNA expression to directly control the protein secretion post-transcription.

3.3.1 Histone Methylation

A study conducted by Yasuto Araki et al. 2016, attempted to study the involvement of

epigenetic mechanisms, especially histone methylation, in regulating the Matrix Metalloproteinase Gene in Rheumatoid Arthritis. As described earlier, MMPs are strong biomarkers for RA, as they are proteins responsible for cartilage damage, tissue repair and wound activity in the Synovial Fibroblasts. In Rheumatoid Arthritis, the activation of the genes regulating the production of this protein is induced by IL-6. The study procured knee tissue samples of RA or OA patients undergoing knee replacement surgery. The tissue samples were studied and cultured before being analyzed. Their study showed that MMP-1, MMP-3, MMP-9 and MMP-13 genes are upregulated in RASFs. The Histone H3 trimethylated at lysine-4 levels were elevated (H3K4me3), while a pattern showed reduced levels or suppression of H3K27me3.

This study supported the idea that histamine methylation regulates genes that result in protein production, which controls inflammation. The evidence for this lies in the four classes of MMPs that were upregulated, each resulting in joint damage that leads to inflamed joints in RA. The trimethylation of some histone-3 lysines, which include H3K4me3, is usually associated with an open chromatin structure and the “transcriptionally” active genes, which could explain why these are upregulated to promote inflammation further. However, methylation at H3K9 is usually associated with the silencing of genes, which could explain why there are suppressed levels, as inhibition of inflammatory genes is not desired (Kouzarides, T. 2007). STAT-3, a transcription factor of IL-6, was found bound to some of the MMP gene promoter regions, which would also explain their activation. (Araki, Y. et al, 2016). Therefore, we see the recurring pattern in all RA patients, where H3K4 is hypermethylated, especially in the MMP promoter region.

3.4 RA Mechanism of Epigenetic Transgenerational Inheritance

There are very few studies looking at this particular inheritance mechanism in RA. However, the mechanism of transmission of epigenetic changes should still apply, and those mechanisms will be discussed in this section.

3.4.1 Transgenerational Inheritance of DNA Methylation

Animal models of stress (Pachierotti F. et al., 2015) have shown that methylation is altered in maternal IL6 and conserved for three consecutive generations. IL6 is implicated in the pathogenesis of RA, and this study may provide evidence for the conservation of IL6 dysregulation in immune disease states. For over 30 years, DNA methylation has been one of the most studied epigenetic areas. Jirtle and Skinner, in 2007, used mouse models to investigate how changes to a maternal diet alter epigenetic patterns in successive generations. Pregnant mice were fed a methyl-rich diet, changing methylation patterns until the F2 generation. (Jirtle and Skinner, 2007.) This altered methylation pattern increased their susceptibility to infections, thus showing a predisposition to disease based on inherited methylation changes. A similar study conducted by Radford et al., 2014 investigated the DNA methylation paternal inheritance. Male mice were fed a low-protein diet, and methylation in sperm cells was altered compared to controls. (Radford et al., 2014) This methylation change was conserved in offspring, and offspring showed phenotypic differences, which caused health and developmental changes.

3.4.2 Transgenerational Inheritance of microRNAs

Although limited, studies have been conducted on the transgenerational epigenetic inheritance of microRNAs in mice. Male mice were exposed to chronic stress and had an altered miRNA profile compared to their control counterparts. They produced offspring with similar miRNA differences and who had reduced hypothalamic-pituitary-adrenal axis stress reactivity.

Male mice exposed to chronic stress had specific increased miRNAs, due to which their offspring had reduced hypothalamic-pituitary-adrenal stress axis reactivity. To ensure that the miRNA was the factor that led to epigenetics developmental difference, the scientists injected the nine microRNAs that are commonly increased in stressed parents into single-cell zygotes. The results showed reprogramming in the offspring, which led to the degradation of the stored maternal RNA and instead showed the transmission of the sperm miRNA (Rodgers, A. et al., 2015).

Lai, KP. et al. 2022 studied miRNA dysregulation due to hypoxia in fish of the species *Oryzias melastigma*. The F0 generation of fish was hypoxia-induced, and their RNA was sequenced. The inheritance was noted by observing the ovarian development of the offspring, which was also found to be impaired, explaining that it would hence be possible that all offspring in the lineage would develop hypoxia. They did this by small RNA sequencing and noticed that between the results of the F0 generation and F2 generation, there were four common dysregulated miRNAs, especially those that heavily contribute to reproduction. This paper helps us understand that in many diseases, especially those that dysregulate miRNA levels, these dysregulations can be inherited across generations.

The paper by Stuppia L. et al., 2015 showed a clear modification in the epigenetics of the sperm of patients with diet-induced diabetes. This resulted in an impact on the development of the zygote of their offspring. Obesity was shown to be passed down two generations due to altered miRNA, making these patients more susceptible to pathologies as these were acquired from the paternal environment. This study explains how environmental factors can cause autoimmune diseases and alter germ cell epigenetics- which impacts offspring development.

3.4.3 Transgenerational Inheritance of Histone Modifications

While this area is yet to be researched further, a few experiments in animal models show evidence for transgenerational inheritance of histone modification. The research experiment (Maamar M. et al., 2019) was carried out to investigate how environmental toxins that impact epigenetics are passed across generations. The study used a mouse model, while the toxin the model was exposed to was the agricultural pesticide DDT and fungicide vinclozolin. The results investigated the histone retention sites and genetic differences between exposed gestational female rats (F0) and their F3 lineage. The analysis showed that the H3 histone was highly conserved, with almost perfectly reproducible results, with over 90% of common histone retention sites and only 10% variation. This was one of the first studies showcasing evidence for histone retention of environmentally induced epigenetic alterations, which is usually observed in the cause of RA, which, although autoimmune, is also heavily influenced

by external factors. The study also contributes to epigenetics, showing how "transgenerational alterations in DNA methylation and non-coding RNA" in sperm or early development stages appear to be epigenetically inherited.

Salgado L. et al., 2022 examined how histone modifications in Zebra Fish were inherited across generations. Both contribute to the overarching idea that there is evidence that histone modifications influence DNA methylation patterns and vice versa. Hence, if DNA methylation, acetylation and other such modifications have evidence of being inherited, histone modifications are likely to be conserved.

4. Discussion

While epigenetics is a relatively recent field still heavily under research, its association with the pre-existing knowledge of genetics opens a vast scope of understanding. There remains much potential in the field that may draw scientists to focus on it due to the possibility of prospects. All the papers studied and surveyed in this review pointed to the idea that any epigenetic dysregulation or fluctuations resulted in inflammation. This reinforces the idea that epigenetics is involved in the progression of RA, considering that extreme inflammation is the primary mechanism that causes joint damage. Similar tumour-related diseases, such as cancer, are also reliant on such a pro-inflammatory response for their pathogenesis. Hence, treatments developed to reduce the effects of epigenetic modifications contributing towards inflammation could be a significant contribution.

Evidence for DNA methylation alterations in RA shows the modification results in inflammation via overexpression of pro-inflammatory cytokines, immune cell dysfunction, and increased Citrullination. This is done by increased methylation, a regulatory mechanism associated with reduced expression, of immune-specific cells and protein receptors such as CTLA-4 responsible for maintaining immune homeostasis. The increased methylation decreased this gene's expression, reducing the production of receptors involved in maintaining the ideal immune cell environment, leading to inflammation. In Citrullination, the evidence above explained how hypomethylation of the promoter region of a gene, which controls the production of PADs, increased Citrullination. As a byproduct, Citrullination increases the concentration of anti-citrullinated protein antibodies responsible for chronic inflammation and joint damage.

The information in the section about microRNAs in RA and Table 1 both reveal that the microRNA dysregulation that occurs in RA can also be seen working towards promoting an inflammatory response. In angiogenesis, where there is a formation of new blood vessels. In order for inflammation to occur at a fast pace, increased blood flow is required. Faster blood flow allows the transportation of inflammatory mediators and immune cells. Hence, we see the body undergo a mechanism to increase vasculature and adapt to the need for more blood flow to aid the inflammation process.

Similarly, in the bone and joint destruction process, we do not see the osteoclasts as dysfunctional in all cases. Inflammation at sites near the osteoclasts is causing them to become dysfunctional. In this case, the miRNAs do not directly influence the progression of

RNA but are regulated in a way that usually promotes inflammation, which increases the pathogenesis of RA.

As for Histone Modifications, we also see a similar pattern where the modifications are regulated to promote an inflammatory response. Generally, from the results, histone acetylation promotes gene activation due to its role in opening the chromatin, allowing easier access to transcription factor genes. Hence, we see hyperacetylation of the IL-6 gene promoter region, a proinflammatory cytokine that increases inflammation when activated. However, we see decreased acetylation, or hypoacetylation, of the FOXP3 gene promoter region, which is known to regulate immune T-Reg cells. The hypoacetylation, which leads to reduced expression of these transcription factors, eventually leads to immune dysfunction due to improper regulation of immune cells. A similar trend is seen in histone methylation and ubiquitination, where gene promoter regions important for the production of proteins in the joints/bones responsible for structural damage and erosion, such as MMPs, were found to be upregulated, specifically in the SFs. This provides evidence for the conclusion that while the methylation patterns, miRNAs and modifications may have different roles and impacts on other organ systems, in the case of rheumatoid Arthritis, they are all regulated in a manner that provides an inflammatory pathway to ensure the pathogenesis of RA.

The section on the RA mechanism of transgenerational epigenetic inheritance outlines ground-breaking studies that successfully provided evidence for the inheritance of these epigenetic modifications across generations. This leads to the understanding that by epigenetic modifications being more susceptible to inheritance, there exists a significant risk suggesting that autoimmune diseases are more likely to be inherited - due to the inheritance of the disease and the inheritance of epigenetic modifications that contribute to the pathogenesis of the disease. Hence, in conducting research at an epigenetic level, disease prevention can be carried out at a molecular level, reducing its chances of reaching offspring. A discovery as such would be a huge breakthrough, considering the significant physical and financial burden that auto-immune diseases pose, primarily due to the limited cures and medical treatments available. Additionally, research in the field of epigenetics is also currently minimal, niche and specialized. However, modern technological and biological advancements are now finding epigenetics to be key biomarkers and regulators that, if understood, can have immense potential.

This paper explores a new argument regarding the transgenerational inheritance of epigenetic dysregulation, specifically in RA. Due to the complex idea involving newer fields of science, this topic needs more primary studies for this hypothesis, making this review something that could lead to more extensive findings. The primary purpose of this review is to explain a hypothesis that could be backed by research in the future.

However, the previous studies and reviews examined to draw up this paper have limitations. Due to the brevity of the paper, only about 30-40 available papers were surveyed, with a capped timeline of those being from the year 2000 onwards. This timeline was maintained to standardize the material used and due to the lack of studies in the field of epigenetics before the early 2000s. Additionally, all the papers read were limited to those written or translated

into English, which could be a limitation. Overall, a study in epigenetics is generally also a limitation we faced writing the paper due to the lack of studies conducted in this field. Hence, this review is crucial to further research in epigenetics.

Many similar future studies can be explored based on reviews such as this. For instance, conducting epigenetic transgenerational studies in mouse models like the one cited about the stress tests. As it is hard to replicate a study of this nature in a cell culture set-up, the progression of methylation/epigenetic markers across culture passages could be noted. Further, for the field of human epidemics, a study noting that members of the same family having RA could be involved in a study which conducts RASF sequencing to see if there are similar epigenetic patterns.

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