Epigenetics meets Rheumatology: Beyond DNA

Sabry A. Shoeib (MD)[1], Alaa E. Abd El Hamid (MD) [1], Emad M. El Shebeiny (MD) [1] and Sameh Y. El Hallab (MBBCH) [2]

[1]Internal Medicine Department, Faculty of Medicine, Menoufia University, Menoufia Governorate, Egypt
[2] Resident of Internal Medicine Department, Samanoud Hospital, Gharbeya Governorate, Egypt
samehusif@yahoo.com

Abstract: Objectives: The aim of this work was to illustrate the relation between the epigenetics and rheumatic diseases and the novel epigenetic therapies. **Data Sources:** Data sources were obtained from the Medline databases (Pub Med, Science Direct, and Medscape). **Article Selection:** This article studied the epigenetics role in rheumatic diseases and the recent epigenetic therapies. **Data Synthesis:** Short reviews were performed in the pathophysiology of the rheumatic disorders in association with epigenetic modifications. **Conclusion:** Despite the epigenetic mechanisms are involved in many rheumatic diseases, yet enhanced understanding of the reversible epigenetic alterations that change gene expression could help in paving the way for prospective treatments of several rheumatic diseases.

[Sabry A. Shoeib, Alaa E. Abd El Hamid, Emad M. El Shebeiny and Sameh Y. El Hallab. **Epigenetics meets Rheumatology: Beyond DNA.** *Stem Cell* 2017;8(4):67-77]. ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 13. doi:10.7537/marsscj080417.13.

Key words: epigenetics, genetics, rheumatology.

1. Introduction:

The function and the morphology of the variable organism cells are significantly different although they have a similar Deoxyribonucleic acid (DNA) that can be explained by the characteristic gene expression, which is continued across development and can be sustained with recurrent cell divisions. The heritable informations that pass through cell divisions are necessary for gene expression maintenance to ascertain their propagation in every daughter cell and these informations are epigenetics **[1].**

In 1940, the father of epigenetics Conrad Waddington explained the meaning of epigenetics as on top of or upon genetics. He proposed the epigenetic landscape for understanding cellular development of the unipotent well-differentiated cells from the pluripotent stem cells. Recently, epigenetics definition includes the non-sequence inheritance through cell divisions **[2].**

The epigenome interprets the organism ability to adapt through expression of many phenotypes as a response to multiple environmental stimuli. The exceptional gene expression program of every cell type shows its functional identity by the epigenome, throughout development or disease. The epigenome consist of series of multiple chemical tags, which act as modifiers of the DNA and its structures that is nucleotide sequence independent. The DNA genetic information within the cells of every organism is defined completely by the genome. The complicated alterations related to DNA are comprised by the epigenome leading to the characteristic cellular identity with development **[3].**

2. Materials and Methods:

Data Sources:

We reviewed papers on the influence of epigenetic modifications on rheumatic diseases from Medline databases, which are (PubMed, Science Direct, and Medscape) and from materials available on the Internet. We used epigenetic/ genetics/ DNA methylation/ histone modifications/ epigenetic mechanisms and rheumatology/ chromatin remodelling. In addition, we examined references recruited from the specialist databases EMF-Portal (http://www.emf-portal.de).

Article Selection:

All the reviews were independently assessed for any updated information about the role of epigenetics in rheumatic diseases. They were included if they fulfilled the following criteria:

Inclusion criteria of the published studies:

-Published in English language.

-Focused on the epigenetics role in rheumatic diseases.

-Discussed the link between epigenetic mechanisms and rheumatology and its therapeutic role in rheumatic diseases.

-If a study had several publications on certain aspects, we used the most recent publications giving the best relevant data.

Data Synthesis:

Short reviews are conducted on different rheumatic diseases associated with epigenetic alterations.

3. Results:

The initial search presented 286 articles, papers,

and journals about the title of the article with the key words mentioned; extraction was performed, including assessment of quality and validity of papers. 127 published articles met the inclusion criteria and are assessed for internal validity. Studies concerning with rheumatic diseases associated with epigenetic changes were collected; each study was reviewed independently; obtained data is rebuilt in new language and arranged in topics through the article.

Table 1: The Posttranslational modifications of different histones [30].

Position	Amino acid	Posttranslational modification	outcome on transcriptional state of chromatin
Histone H3			
4	K	Mono-methylation	Activation
		Di-methylation	Activation
		Tri-methylation	Activation > repression
		Acetylation	Activation
9	K	Mono-methylation	Activation
		Di-methylation	Repression
		Tri-methylation	Repression
		Acetylation	Activation
27	K	Mono-methylation	Activation
		Di-methylation	Repression
		Tri-methylation	Repression > activation
		Acetylation	Activation
Histone H4			
5	K	Acetylation	Activation

K: Lysine amino acid

Table 2: Different targets for organ-specific and systemic rheumatic diseases [31].

Type	Disease	Main affected organ (s)	Cell type
1) Systemic	**SLE**	Skin, joints, heart, brain, kidney, others	T cells
	RA	Joints, skin, lungs, heart and blood vessels, others	RASFs Peripheral blood mononuclear cells CD4 T cells
	Scleroderma	Skin, intestine, lung	Dermal fibroblast
2) Organ-specific	**Multiple sclerosis**	Brain, spinal cord (CNS)	T lymphocytes PSS fibroblasts

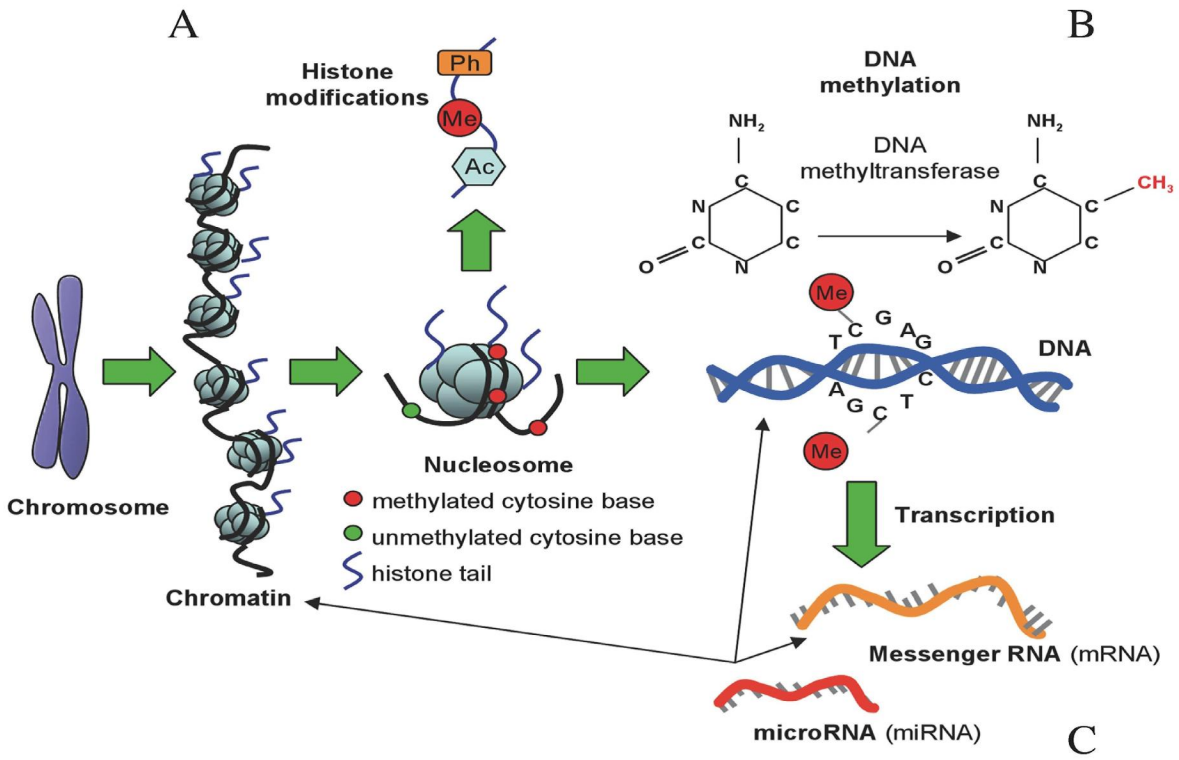
SLE: Systemic lupus erythematosus; RA: Rheumatoid arthritis; RASFs: Rheumatoid arthritis synovial fibroblasts; CD4: cluster of differentiation 4 cells; CNS: Central nervous system; PSS: Progressive systemic sclerosis.

Table 3: Concordance rate of epigenetic rheumatological disorders in monozygotic twins [32].

Disease	MZ CR	Examples of Epigenetic Deregulation Events
1) Systemic lupus erythematosus.	**25%**	Global DNA hypomethylation Focal hypomethylation DNMT1 down-regulation Global histone hypoacetylation Focal hypoacetylation
2) Rheumatoid arthritis.	**15%**	Global DNA hypomethylation Focal hypomethylation Focal hypermethylation
3) Multiple sclerosis. **4) Sjogren syndrome.** **5) Scleroderma.**	**25%**	Focal hypomethylation Focal hypermethylation DNMT1 over expression Global DNA hypomethylation Focal hypermethylation
6) Ankylosing spondylitis.	**50%**	Global hypomethylation

MZ: monozygotic twins; CR: concordance rate; DNA: Deoxyribonucleic acid; DNMT1: DNA methyltransferase 1.

[1]

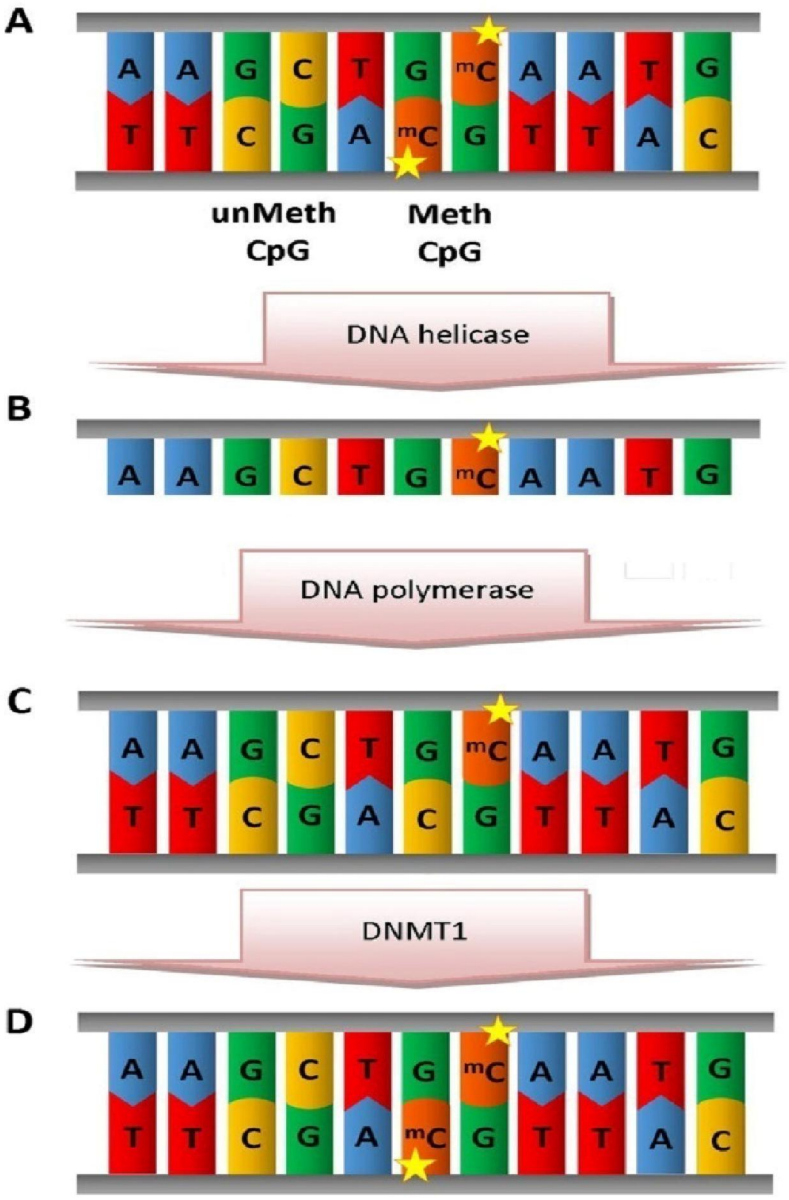


Types of epigenetic modifications.

(A) Histones undergo methylation (Me), acetylation (Ac) and phosphorylation (Ph). They go through transcriptional regulation. **(B)** DNA molecules are methylated by methyl group adding to carbon position 5 on cytosine bases that catalyzed by DNMTS which maintain repressed gene activity. **(C)** Translation of MRNA into a protein that microRNAs can repress it **(Relton, 2010).**

Figure (1): Types of epigenetic modifications.

[2]

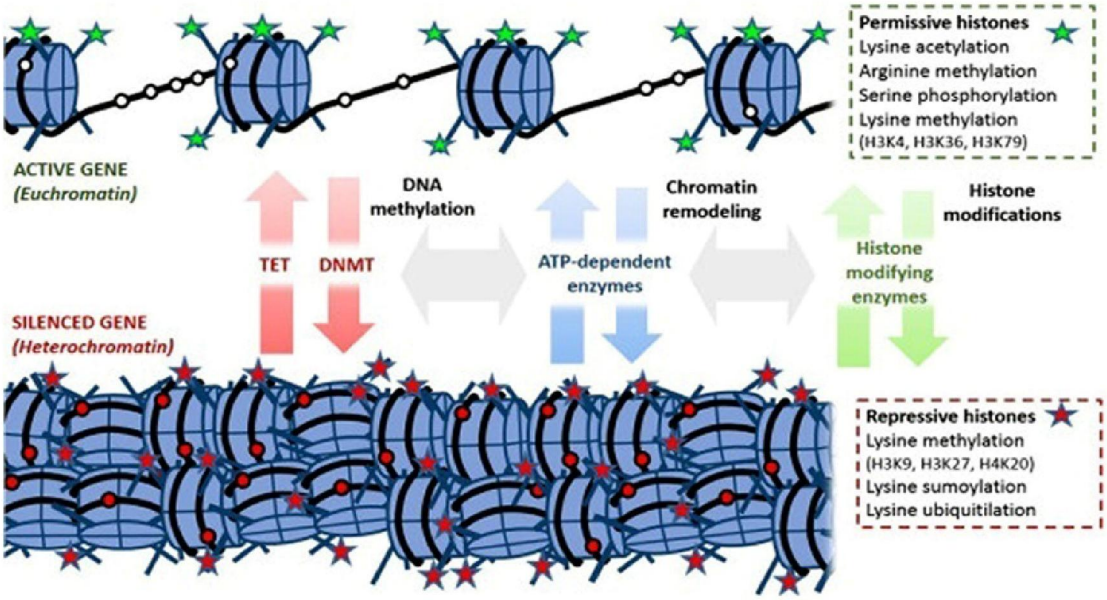


Replication process of the methylated DNA.

CpG sites nature is fundamental for their inheritance **(A)**. With replication, every separated strand carries single methylated cytosine **(B)**. The daughter hemi- methylated DNA identification **(C)** on the new strand by DNMT1 that methylates CpG sites using the old one as a template **(D) (Bégin and Nadeau, 2014).**

Figure (2): Replication process of the methylated DNA.

[3]

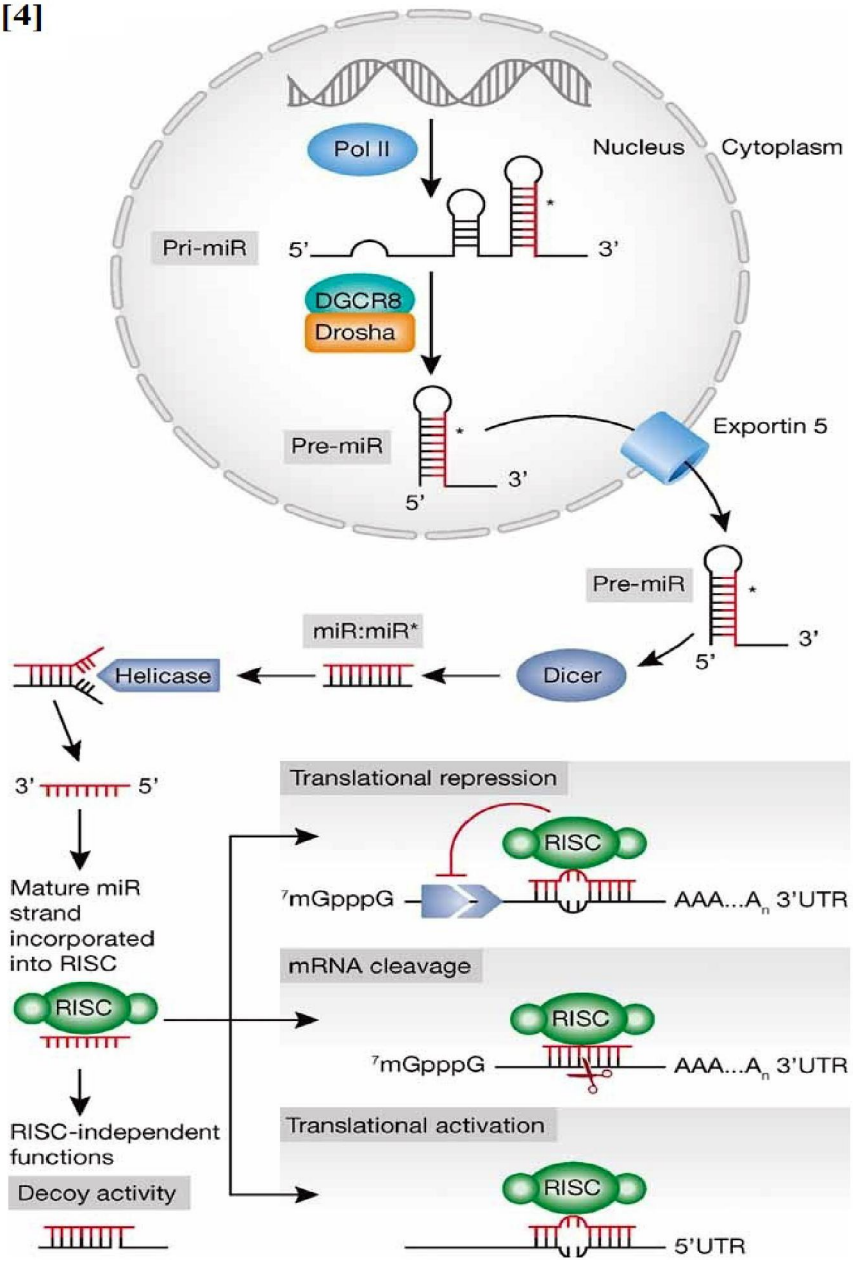


Euchromatin and heterochromatin modifications.

Loose chromatin encourages gene transcription in the euchromatin and condensed chromatin inhibits transcription in the heterochromatin. Different enzymes have the ability to govern the epigenetic regulation without interference in the cooperativity and interaction between the different epigenetic modifications. DNMT =DNA methyltransferase, TET=Ten-eleven translocation dioxygenase, repressive histone modifications (red stars), DNA methylation (red circles), Unmethylated CpG islands (blank circles), permissive histone modifications (green stars) **(Bégin and Nadeau, 2014).**

Figure (3): Euchromatin and heterochromatin modifications.

[4]

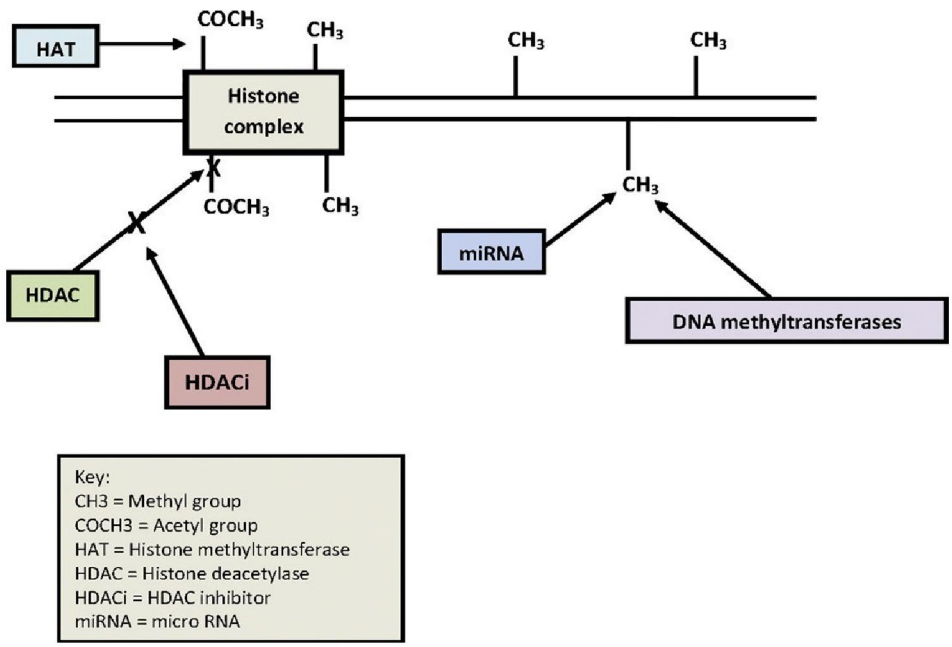


Mechanism of action of miRNA.

MiRNA undergoes nuclear processing by means of Drosha-DGCR8 complex to generate pre-miRNA that is later processed in cytoplasm by Dicer and gets incorporated in RISC. The RISC- miRNA complex suppresses expression by blocking translation or mediating mRNA degradation **(Iorio and Croce, 2012).**

Figure (4): mechanism of action of miRNA.

[5]



Epigenetic targets for management of rheumatic and autoimmune disorders .

HAT acetylates lysine residues while HDAC delete acetyl groups and blocked by HDACi acting as targets for epigenetic therapy **(Ooi et al., 2007).**

Figure (5): Epigenetic targets for management of rheumatic and autoimmune disorders.

4. Discussion:

DNA methylation

Methylation of DNA arises from the response to multiple cellular stressors and signal by specific enzymes that are called DNA methyltransferase. These enzymes act by methyl group adding to position 5 of the cytosine residues at the cytosine guanine dinucleotide (CpG) sites, which consist of the dinucleotides cytosine and guanine linked by phosphodiester bond. The significant regulatory sites such as the promoter or enhancer regions have multiple CpG sites that may accumulate to form the CpG islands **[4].**

The interaction between epigenetic mechanisms and DNA methylation is reversible such as histone modification, which can alter DNA methylation. The Methyl CpG binding proteins may participate, with the transcription factors, in promoted access to binding sites through recruitment by the Methylated DNA. Methyl CpG binding protein for example Methyl CpG binding protein (MeCP2) had the ability to recruit the histone modifying enzymes adding another state of epigenetic modifications **[5].**

De novo DNA methylation during epigenetic silencing in stem cells Studies is to be preceded by chromatin inactivation by chromatin and histone modifying enzymes. DNA methylation is not only considered as an epiphenomenon of other epigenetic mechanisms because it has a particular importance in this field as DNA methyltransferase mutants shows many defects, including reduced stability of genome, aberrant gene expression, and activation of mobile DNA elements **[6].**

Histones modifications

Histones covalent modifications are the second level of epigenetic mechanisms that may be inherited across successive cell divisions. Chemical histone modifications at the terminal tails of histone proteins were noticed during studying the starring role of chromatin in transcription. The relationship between gene expression and histone modification is considered as a universal truth. For example,

acetylation of histone H2A at position K119 lead to repression of transcription while methylation of H3 K4 lead to activation of transcription **[7].**

Histones modifications by special enzymes include methylation and acetylation of lysine, phosphorylation of threonine and serine as well as ribosylation of lysine and ubiquitination of lysine. Epigenetic histone tales modifications impart the nucleosome surface chemical information and memory **[8].**

Chromatin remodelling

Chromatin is a complex of DNA and nucleic proteins seated in the nucleus as a central target of epigenetic modifications. Two dimers of H3 and H4 with two dimers of H2A and H2B constitute the histone octamer. The chromatin types are the active euchromatin and the inactive heterochromatin. The inactive heterochromatin is dense while the active euchromatin with permissive histone modifications had a loose structure **[9].**

Deoxyribonuclease (DNAse) sensitivity assay is a technique used for examination of DNA sensitivity to the enzyme DNAse I to reveal the open or closed state of the chromatin nearby a specific gene. This procedure rely on the fact that DNAse I degrades closed DNA less quickly than open DNA, so termed DNAse I hypersensitivity site (DHS). Moving or ejecting nucleosomes by Histones and Adenosine Triphosphate (ATP)-dependent chromatin remodeling result in a dynamic regulation of Chromatin **[10].**

Ribonucleic acid (RNA) silencing

Non-coding RNA (ncRNAs) refer to a widely expressed group of RNAs lacking protein-coding role. Growing belief supports the ncRNAs fundamental role in chromatin development, modifications, and regulation. Non-coding RNAs can be sorted into the long regulatory, the mid- size, and the short ncRNAs which involve the endogenous small interfering, microRNAs (miRNAs) and Piwi-interacting **[11].**

MiRNAs are small sized non-coding single-strand RNA of 19–22 nucleotides in length regulating gene expression at the post-transcriptional level. They are generated in nucleus by RNA polymerase II which subsequently split by Drosha known as RNAse III. Exportin 5 transport them to the cytoplasm to be processed by Dicer into mature miRNA duplexes; they segregate into single strands at the core of the RNA-induced silencing complex (RISC) by argonaut proteins for generating miRNAs. MiRNAs mostly bind to the untranslated region (UTR) of the targeted messenger RNA (mRNA) result in either mRNA translation or repression or degradation **[12].**

Epigenetics in the pathogenesis of rheumatic diseases

Etiology of Systemic lupus erythematosus (SLE) includes both epigenetic and genetic modifications with environmental factors as chemicals exposure and viral infections resulting in autoimmune response. SLE cases are characterized by alterations in behavior of several immune cells with abnormal signaling pathway and disturbed cytokines level **[13].**

The increased proinflammatory cytokines concentrations are the reason for hyper-reactivation of the synovial cells. Osteoclasts are accused of bone destruction while the Rheumatoid arthritis synovial fibroblasts (RASFs) are considered as the principle effector cells damaging cartilage **[14].**

DNA methyltransferases (DNMTs)

Global methylation phenomenon of DNA is maintained by methylating enzymes as the DNA methyltransferases, which affect S-adenosyl methionine (SAM) methyl group transfer to cytosine for developing 5-methyl-cytosine and S-adenosyl homocysteine (SAH) such as DNMT1 during cellular division and growth, DNMT2 and DNMT3A and 3B in early development. SLE patients showed low DNMT1 activity in the cluster of differentiation (CD4+) cells with low DNMT mRNA levels **[15].**

The aggressive Rheumatoid arthritis synovial fibroblasts (RASFs) are associated with less DNMT1 levels and more alterations in global DNA methylation than the healthy ones that raise cytokines and metalloproteinases expression. Treating synovial fibroblasts from healthy donors with DNA hypomethylating drugs such as 5-aza-2-desoxicytidine resulted in accepting them an aggressive behavior **[16].**

Histone deacetylase enzymes (HDAC)

Histone modifying complexes play a vital role in addition or removal of the various chemical elements on histones such as histone deactetylase (HDAC) which remove acetyl groups developing condensed and silenced chromatin and histone acetyl transferase (HAT) by acetyl groups addition on histone residues making chromatin less compact and active. Covalently modified amino acids on histone tails may influence chromatin structure and recruit chromatin-remodelling complexes helping target genes for transcription initiation **[17].**

HDAC enzymes affect Toll-like receptor (TLR) signaling raising the proinflammatory mediators' expression by repression of Nuclear factor kappa of B cells (NFkB) inhibitors such as inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-b). HDACs control stability and function of regulatory T cell (Treg) cells throughout forkhead box P3 (Foxp3) transcription factor alteration **[18].**

Epigenetic therapy of rheumatic diseases

Epigenetic treatment fights diseases by microRNAs and histone deacetylase inhibitors (HDACi) that include phenylbutyric acid, The suber-oylanilide hydroxamic acid (SAHA), valproic acid,

and Trichostatin A as well as by DNA methylation inhibitors which include 5-azacytidine, 5-aza-2′-deoxycytidine, Procainamide, Decitabine, Zebularine, and Procaine Hydralazine that reactivate silenced genes by suppression of DNMT enzymes **[19].**

Direct epigenetic drugs effects in the control of a single gene or cell in which dysregulation of transcription takes place may result in decreasing the risky side effects since other irrelevant genes will be spared. Drugs influencing gene transcription create normal transcription and consequent improvement of clinical symptoms **[20].**

DNA methylation inhibitors

The 5-Azacytidine is used for treatment of Myelodysplastic syndrome (MDS) patients, exhibiting demethylating properties with lupus-like disorders that frequently reported after long-lasting administration due to global DNA hypomethylation mechanism while the termination of treatment resulted in symptoms disappearance **[21].**

Methyl-CpG binding proteins (MBD) in SLE cases may sustain hypomethylation of active DNA as MBD2 and MBD4. The demethylating MBD2 mRNA and MBD4 has the ability to increase the transcription level in SLE patients **[22].**

The competitive DNA methyltransferase (DNMT) inhibitor Procainamide is noticed to be associated with lupus as iatrogenic lupus drug as well as Another demethylating drug known as hydralazine suppressing the extracellular-signal-regulated kinase (ERK) signaling with consequent inhibition of DNMT activity that result in DNA hypomethylation which occurs also in idiopathic lupus type **[23].**

Histone deacetylase inhibitors (HDACi)

HDAC inhibitors play a vital role in rheumatoid arthritis treatment that can be explained by the up-regulation of cell cycle inhibitors cyclin-dependent kinase inhibitor 2A (p16INK4) and cyclin-dependent kinase inhibitor 1 (p21Cip1) by histone acetylation and down- regulation of proinflammatory cytokines and their action as chromatin modifiers with the balance involving synovial hyperplasia and oncogenesis due to gene regulation malfunction. Studies showed that topical use of Trichostatin A (TSA) and phenylbutyrate (PB) had induced p16INK4 and p21Cip1 expression in synovium of rats with adjuvant arthritis that did not occur in normal synovial cells by isolation of their RASFs that arrested their proliferation. In vitro administration of topical TSA ointment or PB cream resulted in suppressing the proinflammatory cytokines Interleukin-6 (IL-6) that is implicated in DNA methylation and Tumor Necrosis Factor-alpha (TNF-a) with improvement of joint swelling as well as enhancement of wound healing and further suppression of pannus formation and paw swelling **[24].**

The inhibitory role of HDAC inhibitors such as Trichostatin A (TSA) in rheumatoid arthritis (RA) effector cell types is evident with consequent disease suppression by reducing autoreactive T helper 1 (Th1) and balancing T cells to be protected via elevation of Treg cell effects and T helper 2 (Th2) levels **[25].**

The suber-oylanilide hydroxamic acid (SAHA) and the selective HDAC1 inhibitor Entinostat (MS275) inhibit the proinflammatory cytokines (IL-1beta, TNF-alpha) and have been reported in collagen-induced arthritis to exhibit antirheumatic properties as down-regulation of matrix metalloproteinases (MMP) expression in Synovial fibroblasts with delaying arthritis onset and stopping bone damage **[26].**

A single intravenous infusion of the Histone deacetylase inhibitor Romidepsin (FK228) leads to attenuation of Autoantibody-mediated arthritis as well as inhibition of RASFs propagation in vitro by acting as HDAC1 and HDAC2 inhibitor. Joint destruction and Synovial proliferation in autoantibody-mediated arthritis mice were reported to be improved by systemic treatment with FK228 resulting in cyclin-dependent kinase (CDK) inhibitors up-regulation **[27].**

MicroRNA (miRNA)

CD4+ T cells In SLE patients show high overexpression of miRNA-21 and miRNA-148 while DNMT1 proteins had reduced levels resulting in hypomethylation of DNA and elevated gene transcription of Cluster of Differentiation 20 (CD20) and the autoimmune-related cellular marker Lymphocyte function-associated antigen 1 (LFA-1). These CD4+ T cells also exhibited knockdown of miRNA-126 with autoimmune activity depression by reduced production of immunoglobulin G (IgG) from B cells. Disease-relevant miRNAs direction for rheumatic diseases treatment had been studied in mice with autoantibody-mediated arthritis reporting that up-regulation of the anti-apoptotic protein *B-cell lymphoma 2 gene (Bcl-2)* induced normal apoptosis suppression that could be reversed by action of miRNA and reported also motivating apoptosis via use of the intrarticular miRNA-15a into the synovium **[28].**

One of the most important regulators of cytokine formation is miRNA-146a which is noticed to be elevated in systemic lupus erythematosus such as TNF-a. The miRNA-155 assists Th1 and Th17 development. The CD4+, CD25+, Foxp3+ and T reg cells in a mouse model (MRL/lpr) with SLE exhibited increased level of MiRNA-155, which targets CD62L with low suppressive capacity of the Treg cells. DNMT1 implicate low activity in CD4+ T cells with suppressed translation by miRNA-126 overexpression in SLE resulting in increased B- and T-cell autoreactivity. Consequently, methylation of the immune-related genes *Integrin Alpha (ITGAL)*, which

code for CD11a and Tumor Necrosis Factor Ligand Superfamily Member 7 (TNFSF7) that code for CD70, is declined causing subsequent overexpression **[29].**

5. Conclusion

Epigenetics is the changes of physiological and cellular phenotypic features by environmental and endogenous factors affecting cells ability to read genes independently on DNA sequence changes. Epigenetic modifications by DNA methylation, Histone modification, chromatin remodeling and RNA interference. Epigenetics mechanisms may add chemical groups to DNA and histones by the action of writers, erasers and readers making chromatin more or less accessible to transcription. Epigenetic changes are involved in pathogenesis of many rheumatic diseases by gene silencing or activation that may also develop several autoimmune diseases and cancer. Changes of gene expression by epigenetic modifications are reversible that help establishment of innovative treatments for rheumatic diseases such as histone deacetylase inhibitors, microRNAs, and DNA demethylating agents. Adverse effects of epigenetic therapy by are due to lack of direct targeting of genes. Novel therapies will be more specific and prospective by the suggestion that 50% of promoter regions are situated in CpG islands.

Corresponding Author:
Name: Sameh Y. ElHallab,
Address: Resident of Internal Medicine Department, Samanoud Hospital, Gharbeya Governorate, Egypt
E-mail: samehusif@yahoo.com

References:

1. Adalsteinsson BT and Ferguson-Smith AC. (2014): Epigenetic Control of the Genome Lessons from Genomic Imprinting. Genes (Basel); 5(3): 635–655.
2. Baedke J. (2013): The epigenetic landscape in the course of time: Conrad Hal Waddington's methodological impact on the life sciences. Stud Hist Phil Biol Biomed Sci; 44(4):756–773.
3. Rivera CM and Ren B. (2013): Mapping human epigenome Cell; 155(1):39-55.
4. Wu SC and Zhang Y. (2010): Active DNA demethylation: many roads lead to Rome. Nat Rev Mol Cell Biol.; 11(9):607–620.
5. Murr R. (2010): Interplay between different epigenetic modifications and mechanisms. Adv Genet. 2010; 70:101-41.
6. Rossnerova A, Tulupova E, Tabashidze N, et al. (2013): Factors affecting the 27K DNA methylation pattern in asthmatic and healthy children from locations with various environments. Mutat Res; 741– 742:18–26.
7. Li QJ, Chau J, Ebert PJ, et al. (2007): miR-181a is an intrinsic modulator of T cell sensitivity and selection. Cell; 129:147-61.
8. Turner BM. (2007): Defining an epigenetic code. Nature Cell Biology. 9:2-6.
9. Breton CV, Salam MT, Wang X, et al. (2013): Particulate matter, DNA methylation in nitric oxide synthase, and childhood respiratory disease. Environ Health Perspect; 120(9):1320–1326.
10. Tarantini L, Bonzini M, Apostoli P, et al. (2009): Effects of particulate matter on genomic DNA methylation content and iNOS promoter methylation. Environ Health Perspect; 117 (2):217–222.
11. Place RF and Noonan EJ. (2014): Non-coding RNAs turn up the heat: An emerging layer of novel regulators in the mammalian heat shock response. Cell Stress and Chaperones; 19 (2):159–172.
12. Konsta OD, Thabet Y, Le Dantec C, et al. (2014): The contribution of epigenetics in Sjögren's Syndrome. In Front Genet. ECollection 2014; 5: 71.
13. D'Cruz DP, Khamashta MA, Hughes GR. (2007): Systemic lupus erythematosus. Lancet; 369:587-96.
14. Neumann E, Lefevre S, Zimmermann B, Gay S, Muller-Ladner U. (2010): Rheumatoid arthritis progression mediated by activated synovial fibroblasts. Trends Mol Med; 16:458-68.
15. Deng C, Kaplan MJ, Yang J, Ray D, Zhang Z, McCune WJ, et al. (2001): Decreased Ras-mitogen-activated protein kinase signaling may cause DNA hypomethylation in T lymphocytes from lupus patients. Arthritis Rheum; 44:397-407.
16. Karouzakis E, Gay RE, Michel BA, Gay S, Neidhart M. (2009): DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum; 60:3613-22.
17. Zeng L, Zhang Q, Li S, et al. (2010): Mechanism and regulation of acetylated histone binding by the tandem PHD finger of DPF3b. Nature; 466 (7303):258–262.
18. Beier UH, Akimova T, Liu Y, Wang L, Hancock WW. (2011): Histone/protein deacetylases control Foxp3 expression and the heat shock response of T-regulatory cells. Curr Opin Immunol; 23(5):670-8.
19. Nandakumar V, Vaid M, Katiyar SK. (2011): Epigallocatechin-3-gallate reactivates silenced tumor suppressor genes, Cip1/p21 and p16INK4a, by reducing DNA methylation and increasing histones acetylation in human skin cancer cells. Carcinogenesis; 32:537-44.

20. Janson PC and Winqvist O. (2011): Epigenetics - the key to understand immune responses in health and disease. Am J Reprod Immunol; 66 (Suppl.1):72-4.
21. Kaminskas E, Farrell A, Abraham S, Baird A, Hsieh LS, Lee SL, et al. (2005): Approval summary: azacitidine for treatment of myelodysplastic syndrome subtypes. Clin Cancer Res; 11:3604-8.
22. Detich N, Theberge J, Szyf M. (2002): Promoter-specific activation and demethylation by MBD2/demethylase. J Biol Chem; 277:35791-4.
23. Chang C and Gershwin ME. (2010): Drugs and autoimmunity contemporary review and mechanistic approach. J Autoimmun; 34: J266-75.
24. Chung YL, Lee MY, Wang AJ, Yao LF. (2003): A therapeutic strategy uses histone deacetylase inhibitors to modulate the expression of genes involved in the pathogenesis of rheumatoid arthritis. Mol Ther; 8:707-17.
25. Tao R, de Zoeten EF, Ozkaynak E, Chen C, Wang L, Porrett PM, et al. (2007): Deacetylase inhibition promotes the generation and function of regulatory T cells. Nat Med; 13:1299-307.
26. Choo QY, Ho PC, Tanaka Y, Lin HS. (2010): Histone deacetylase inhibitors MS-275 and SAHA induced growth arrest and suppressed lipopolysaccharide-stimulated NF-kappaB p65 nuclear accumulation in human rheumatoid arthritis synovial fibroblastic E11 cells. Rheumatology (Oxford); 49:1447-60.
27. Nishida K, Komiyama T, Miyazawa S, Shen ZN, Furumatsu T, Doi H, et al. (2004): Histone deacetylase inhibitor suppression of autoantibody-mediated arthritis in mice via regulation of p16INK4a and p21(WAF1/Cip1) expression. Arthritis Rheum; 50:3365-76.
28. Nagata Y, Nakasa T, Mochizuki Y, et al. (2009): Induction of apoptosis in the synovium of mice with autoantibody- mediated arthritis by the intraarticular injection of double-stranded MicroRNA-15a. Arthritis Rheum; 60:2677-83.
29. Lu Q, Kaplan M, Ray D, Zacharek S, Gutsch D, Richardson B. (2002): Demethylation of ITGAL (CD11a) regulatory sequences in systemic lupus erythematosus. Arthritis Rheum; 46:1282-91.
30. Lennartsson A and Ekwall K. (2009): Histone modification patterns and epigenetic codes. Biochim Biophys Acta. 2009; 1790:863–868.
31. Arora-Singh RK, Assassi S, del Junco DJ, Arnett FC, Perry M, Irfan U, et al. (2010): Autoimmune diseases and autoantibodies in the first degree relatives of patients with systemic sclerosis. J Autoimmun; 35:52-7.
32. Qi Q, Guo Q, Tan G, Mao Y, Tang H, Zhou C, et al. (2009): Predictors of the scleroderma phenotype in fibroblasts from systemic sclerosis patients. J Eur Acad Dermatol Venereol; 23:160-8.

6/19/2017