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RESEARCH ARTICLE

MIRNA 146 AND ITS ASSOCIATION WITH AUTOIMMUNE DSEASES

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Abstract

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression at the post-transcriptional level through base-pairing predominantly with a 3'-untranslated region of target mRNA, followed by mRNA degradation or translational repression. Totally, miRNAs change, through a complex regulatory network, the expression of more than 60% of human genes. MiRNAs are key regulators of the immune response that affect maturation, proliferation, differentiation, and activation of immune cells, as well as antibody secretion and release of inflammatory mediators. In this review, we generally discuss miRNAs, its types and its role in the regulation of the immune system and the autoimmune inflammatory process, focusing on the participation of miRNA-146 in the development of multiple sclerosis (MS), Rhumatoid arthritis and Type-I diabetes mellitus. Disruption of this regulation may lead to the development of various pathological conditions, including autoimmune inflammation. Special attention is given to the role of miRNA-146 in the autoimmune inflammation in multiple sclerosis, Rhumatoid arthritis and Type-I diabetes mellitus. This study concluded that, dysregulation of miR-146 and its target genes was one of the main causes for many autoimmune diseases; our findings indicate a significant association of decreased miR-146 expression and the sustained immune imbalance in multiple sclerosis, Rhumatoid arthritis and Type-I diabetes mellitus.

Introduction:-

MicroRNAs (miRNAs) are small (~22 nucleotides) noncoding RNA sequences that inhibit gene expression of specific mRNA targets[1]. Awareness of the fact that more than 80% of the genome has a specific biological function primarily associated with regulation of the expression of protein-coding genes became one of the most important results of the Encyclopedia of DNA Elements project, which was aimed at deciphering the functional part of the genome. The most commonly identified functional elements were genes encoding various regulatory RNAs, including miRNAs (short, 19 to 24 nucleotide, single-stranded RNA molecules), which are key regulators of various biological processes at the post-transcriptional level [2]**.** Cell mRNAs, have unique, various expression patterns and have an effect on many cell strategies and developmental pathways **[3, 4]** Most miRNA genes are transcribed with the aid of RNA polymerase II (Pol II), with the lengthy major transcript, termed pri-miRNA, harboring a hairpin

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structure, which involves the miRNA sequence. Whereas many of these genes are transcribed as intronic clusters inside protein-coding pre-mRNAs, others can be transcribed as impartial gene units, or be encoded inside lengthy non-coding RNAs **[2, 3]**

MiRNAs are highly conserved molecules. Evolutionary related miRNAs are combined into 239 different families whose members have highly homologous sequences and some common targets [5]. Recent studies have demonstrated that miRNAs are essential for the normal development of various physiological systems in organisms and maintenance of cell homeostasis, while a change in their expression and/or function is associated with the development of many pathological conditions in humans, including oncological, infectious, neurodegenerative, and autoimmune diseases [6]**.** It has been reported that dysregulation of microRNAs (miRNAs) may contribute to the pathogenesis of autoimmune diseases including T1D **[4]** reported that, Type 1 diabetes mellitus (T1D) is a common autoimmune disease mediated by autoimmune attack against pancreatic β-cells. The dysregulation of miR-146 expression may be associated with the ongoing autoimmune imbalance in T1D patients. In this review, we generally discuss miRNAs, its types and its role in the regulation of the immune system and the autoimmune inflammatory process, focusing on the participation of miRNA-146 in the development of multiple sclerosis (MS), Rhumatoid arthritis and Type-I diabetes mellitus.

Mirna definition:

MiRNAs are small non-coding RNAs (ncRNAs), about 20 nucleotides (nt) in length, that adjust gene expression posttranscriptionally through binding to 3` untranslated areas (UTR), coding sequences or 5`UTR of goal messenger RNAs (mRNAs) and main to inhibition of translation or mRNA degradation [7, 8]. It is estimated that miRNAs alter about 30% of the human protein-coding genome .miRNAs manage the expression of genes worried in various biologic processes, such as apoptosis, proliferation, differentiation, and metastasis [9].

Mirnas mechanism of action:

Most miRNAs are encoded by genes located in the introns of protein-coding genes; miRNA genes can also be localized in exons, 5'- and 3'-untranslated gene regions, or intergenic regions **[10].** MiRNA genes are transcribed in the nucleus, primarily by RNA polymerase II, as a primary miRNA (pri-miRNA), which is a long transcript (from a few hundred to tens of thousands of nucleotides). The primary miRNA is then converted into a miRNA precursor (pre-miRNA) by the Drosha-DGCR8 microprocessor complex (canonical pathway) [6]. There are also several other non-canonical pathways of premiRNA production, one of which is the formation of a pre-miRNA during splicing of short hairpin introns (mirtrons), followed by cleaving of pre-miRNA by the Ldbr protein **[11]**. Then, the miRNA biogenesis pathways merge, and the pre-miRNA is processed in the cytoplasm by the Dicer enzyme (RNase III) to form a miRNA duplex, with one of the duplex chains being involved in the formation of the RNA-induced silencing complex (RISC).The following diagram illustrating the biogensis and mechanism of action.

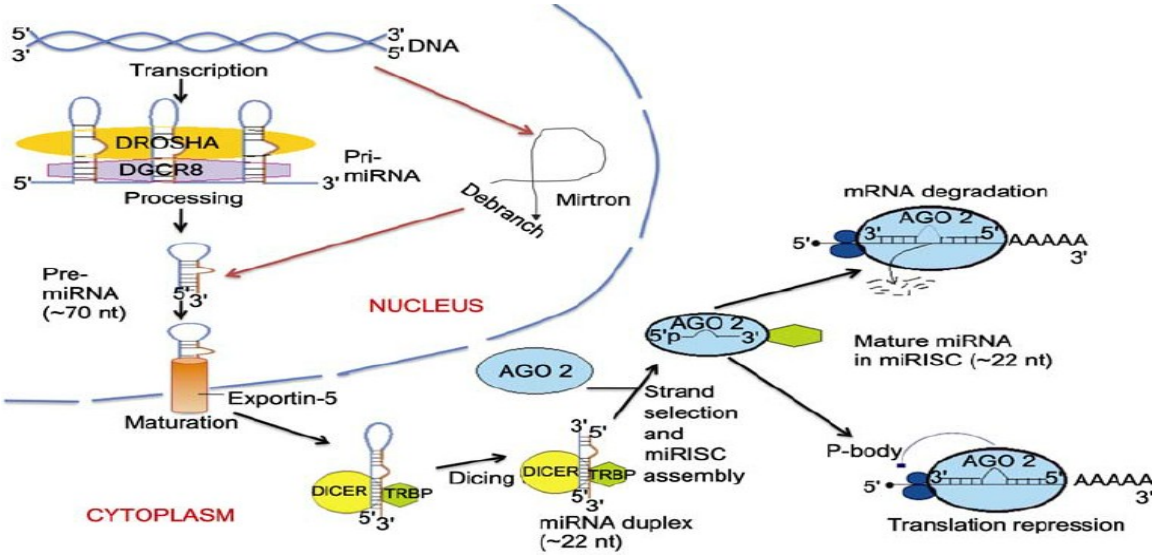

Figure 1:- Schematic diagram of the biogenesis of miRNA and its mechanism of action. In the nucleus the primary miRNAs (pri-miRNAs) are transcribed from the genome by RNA polymerase II which are then processed by

Microprocessor (Drosha and DGCR8) into precursor miRNAs (pre-miRNAs). These pre-miRNAs are then exported into the cytoplasm by a Ran-GTP dependent nuclear transport receptor, exportin 5 where they are further processed into ~22 nucleotide long miRNA duplex by Dicer and its interacting partner, TRBP (TAR RNA Binding Protein). One strand of this miRNA duplex along with Dicer, TRBP and a member of the Argonaute protein fadown-regmily (AGO2) assembles into the miRNA Induced Silencing Complex (miRISC). Mature miRNAs then regulate gene expression by guiding the miRISC to their target complementary mRNA, causing mRNA degradation and repression of translation initiation. Mirtrons are produced from pri-miRNA-sized introns that form a looped intermediate called a lariat, which are then debranched and refolded into pre-miRNAs that enter the canonical biogenesis pathway [12].

A small, 6–8 nucleotide, miRNA fragment, the seed region, is critical for miRNA binding to the target mRNA within the RISC complex. The degree of complementarity between this miRNA fragment and the target mRNA largely determines the mechanism of gene expression regulation. Complete complementary binding between miRNA and mRNA leads to cleavage and degradation of mRNA. In the case of incomplete complementarity between miRNA and a target mRNA, mRNA translation is inhibited at the stage of initiation or elongation, and the mRNA is destabilized due to cleavage of a polyA sequence and is transferred to processing bodies. MiRNA can act at the transcriptional level through regulation of chromatin reorganization **[11, 13].** In most cases, miRNAs reduce the expression level of a target mRNA; but in some cases, binding of miRNAs to certain protein complexes can increase expression of target genes via direct or indirect mechanisms [13]**.**

At present, miRNAs are known to function not only inside the cells, but are also capabale of being secreted into the bloodstream[14]. Like cytokine action, the miRNA function is characterized by degeneracy (redundancy) and pleiotropy; i.e. the expression level of one mRNA can be regulated by many miRNAs, and one miRNA binds to many target mRNAs, which results in the formation of a complex regulatory network. Thus, a change in the expression of one miRNA may lead to changes in the expression profile of many target mRNAs: however, this effect for each individual mRNA will also depend on the influence of other miRNAs [8, 9]. Currently, heaps of miRNAs have been recognized in people and different species, and miRNA on line sequences repositories, such as the miRbase database, are available. Furthermore, modern equipment and software program developed for miRNA goal prediction facilitate research of miRNAs useful network [15, 16].

MicroRNAs in autoimmune diseases:
Psoriasis is the most common persistent inflammatory pores and skin sickness in adults.

miRNA-203 in Psoriasis:
miR-203 considered as the first inflammatory ailment in which miRNAs have been implicated. In 2007, Sonkoly et al. discovered that miR-203, which is expressed in keratinocytes, is upregulated in psoriasis-affected pores and skin in contrast with wholesome human pores and skin or any other persistent inflammatory pores and skin disease [17].

Rheumatoid arthritis (RA):
is a systemic autoimmune ailment characterised through persistent irritation of synovial tissue.

MiRNA-155 and miRNA-146 in Rheumatoid arthritis:
In 2008, three researches stated the first affiliation research between RA and miRNAs. Stanczyk et al. discovered improved expression of miR-155 and miR-146 in RA synovial fibroblasts and RA synovial tissue [18].

Moran et al., (48) have examined that miRNAs control the expression levels of IL-1β in RA. It is well documented that the miR-146a/b monitors the expression of IL-1β along with TNFα induced in RA synovial fibroblast (RASFs).

Rheumatoid arthritis FLS plays a key role in joint destruction and is believed to spread RA to unaffected joints [19]. Roles of several miRNAs in T cell proliferation, apoptosis, and cell cycle of synovial cells and joint destruction are depicted. Importantly, there appears to have a feedback by which miRNAs contribute to inflammatory reactions and synovial phenotype in rheumatoid joints. Through NF-kB pathway, some miRNAs such as miR-146a and miR-155 may stimulate the release of pro-inflammatory cytokines such as TNF-a, IL-1b, and IL-17.These cytokines can induce lymphocytes, resident synovial cells and other inflammatory cells to produce miRNAs that related to disease activity of RA patients [20].

MiR-146a, 155, and 223 have opposite roles in regulating RA pathogenesis. These miRNAs are positively correlated with disease activity through stimulating inflammatory response. On the contrary, they play a role in suppressing joint destruction through suppressing osteoclastogenesis by repressing metalloproteinases. The opposite roles of miRNAs give us a lesson to focus more carefully on therapeutic and diagnostic applications of these miRNAs in RA **[21, 22]**.

Multiple scelerosis (MS):
is a severe chronic autoimmune inflammatory CNS disease associated with a complex of immune-mediated pathological reactions that destruct the myelin sheath of neurons, which eventually leads to irreversible loss of neurological functions and severe disability. Currently, the etiology and pathogenesis of MS are not fully elucidated; however, numerous studies suggest the triggering role of the autoimmune process causing damage to the myelin sheath of nerve cells in the CNS. Activation of anergic T and B lymphocytes in the periphery (outside the CNS) is the first stage in MS immunopathogenesis [9].

Mirna-146 and other miRNAs in Multiple scelerosis:
The relationship between immunopathological and neurodegenerative processes enables use of experimental autoimmune encephalomyelitis (EAE) as the primary animal model for studying the role of miRNAs in MS development [23]. One of the first studies demonstrated that ***MIR155*** knockout mice are resistant to EAE due to reduced differentiation of Th1 and Th17 cells upon autoimmune inflammation [24]**.** Screening studies revealed changes in the expression of many miRNAs upon EAE. For example, 43 miRNAs were identified whose expression in the lymph nodes of EAE rats was higher than that in rats resistant to EAE [25]; 33 of these miRNAs were previously associated with the development of MS and other AIDs. In oligodendrocytes of EAE mice, expression of 56 miRNAs was lower than that in oligodendrocytes of normal mice; the lowest expression level was that of miR-15a-5p, -15b-5p, -20b-5p, -106b-5p, -181a- 5p, -181c-5p, -181d-5p, -320-3p, -328-3p, and -338-3p [24]**.**

Studying the role of miRNAs in the EAE development helped identify specific target genes of some miRNAs and evaluate their involvment in the pathogenesis of the disease. The main method to induce EAE in C57Bl/6 (39) and SJL [26] mice or Dark Agouti and PVG rats was immunization with the myelin oligodendrocyte glycoprotein, proteolipid protein, or their immunogenic peptides in a complete Freund's adjuvant in combination with the pertussis toxin [25]. The studies were performed mainly in cells of the immune system (in particular, CD4+ T lymphocytes) and also in various cells of the nervous tissue. An increase in the expression level of miRNA genes (except miR-20b and miR-132/212) was mostly observed in CD4+ T cells; in nervous system cells, expression of the three miRNAs was reduced, and that of two miRNAs was enhanced. The main targets of miRNAs both in CD4+ T lymphocytes and in nervous system cells were mRNAs of genes of transcription factors and modulators of transcription factor activity and genes of signaling pathway elements and cytokines. It is important to note that targets of miR-29b and miR-20b are mRNAs of the ***TBX21*** and ***RORC*** genes encoding T-bet and RORγt, which are the main transcription factors involved in the differentiation of Th0 cells to Th1 and Th17 cells, respectively. The target of miR-326 is the ***ETS1*** gene encoding a transcription factor that directly controls the expression of cytokine and chemokine genes and is involved in the regulation of differentiation and proliferation of lymphoid cells [10, 11]**.**

Type 1 diabetes mellitus (T1DM):
is an autoimmune disease characterized by autoimmune destruction of pancreatic beta-cells by T lymphocytes and macrophages [27]**.** The disease is usually diagnosed when over 80–90% of beta-cells have been destructed by the infiltrating immune system. T1DM development is slow, providing a potentially long window of time in which it is possible to identify and theoretically treat individuals at risk [27-29]**.**

The first sign of autoimmunity against beta-cells, frequently detectable a few months/years before the appearance of clinical symptoms, is the occurrence of antibodies against beta-cell antigens **[30].** These autoantibodies are used as biomarkers of T1DM risk and are directed against insulin, glutamic acid decarboxylase, zinc cation efflux transporter and tyrosine phosphatases-2 and -2β **[30]**. The presence of more than two of these autoantibodies indicates high risk for T1DM development [27, 31, 32]**.** However, the use of islet autoantibodies as biomarkers of T1DM progression has some limitations, especially because a subset of children with new-onset T1DM is negative for islet autoantibodies [31], and many autoantibody-positive subjects will never develop T1DM [33]**.** Moreover, autoantibodies cannot be used as markers to initiate a potential treatment at earlier stages of the disease when many beta-cells are still present **[33].** Thus, new biomarkers of T1DM are necessary to complement the information obtained from the presence of autoantibodies together with genetic and environmental risk factors [34]**.**

- **miRNA-146 and Type 1 diabetes mellitus (T1DM):**
- Growing evidence suggests that miRNAs also play a key role in immune system functions as well as in beta-cell metabolism, proliferation and death, which are processes involved in T1DM pathogenesis **[35]**. Indeed, IL-1β and TNF inflammatory cytokines were reported to induce miR-21-5p, miR-30b-3p, miR-34, miR-101a and miR-146a-5p expressions in MIN6 cells and human pancreatic islets (Roggli et al., 2010 and Zheng et al., 2015), suggesting that these miRNAs may have a role in cytokine-mediated beta-cell destruction. Some miRNAs seem to modulate mRNA expressions of the major T1DM autoantigens [4].

Yang, Boldin [4] reported that, the association between miRNA expression and serum glutamic acid decarboxylase antibody (GADA) titers was also investigated. They compared with normal controls, there were 26 miRNAs and 1218 genes differently expressed in PBMC of patients with newly diagnosed T1D. The greatest downregulation was for miR-146a (48% decrease; $P < 0.05$). Expression of its target genes, predicted to be tumor necrosis factor receptor-associated factor 6 (TRAF6), B cell CLL/lymphoma 11A (BCL11A), syntaxin 3 (STX3) and numb homolog (NUMB), was upregulated. Moreover, T1D patients on long-course insulin and optimized glucose control had sustained low expression of miR-146. Interestingly, decreased miR-146a expression was significantly associated with high serum GADA titers.

Importance of mirna-146 and polymorphism:

MiRNA-146 is estimated to regulate the expression of more than 60% of protein-coding genes **[36]**; consequently, changes in their expressions have been linked to many diseases, including cancer, endocrine disorders and autoimmune diseases **[34].** MiR-146 was the most downregulated miRNA in the PBMC of patients with newly diagnosed T1D, and this downregulation was independent of hyperglycemia and disease duration. Interestingly, decreased miR-146a expression was associated with high GADA titers [5, 6]. MiR-146a was a regulator that controlled the resolution of T cell responses in T cells lacking miR-146a which was hyperactive in both acute antigenic responses and chronic inflammatory autoimmune responses [4]. Underexpression of miR-146a was relevant to the biological and clinical behavior of systemic lupus erythematosus (SLE) [37].

Rapid changes in miR-146a expression negatively regulated the interleukin-1β-induced inflammatory response in human lung alveolar epithelial cells, [38] and knockout of the miR-146a gene led to myeloid sarcomas and lymphomas in C57BL/6 mice [35]. Lipopolysaccharide has been shown to induce miR-146a expression in a nuclear factor-κB-dependent manner [39]. Therefore, miR-146a is involved in the regulation of inflammatory and/or innate immune pathways.

Genetic polymorphism:-

is defined as the occurrence in the same population of two or more alleles at one locus, each with appreciable frequency, where the minimum frequency is typically taken as 1% [40]. An allele is one of the variant forms of a gene at specific locus on a homologous chromosome. The different forms of the polymorphism (alleles) are observed more often in the general population than mutations. The most common polymorphism in the human genome is the single-nucleotide polymorphism (SNP) [41]. **SNPs** are popular molecular genetic markers in disease genetics studies and pharmacogenomic research. It is a single base change in a DNA sequence, with a normal alternative of two possible nucleotides at a given position. This variation occurs at a specific position in the genome and has allele frequency of 1% or greater [42]. Around 325 million, SNPs have been identified in the human genome, 15 million of which are present at frequencies of 1% or higher across different populations worldwide [43].

The majority of SNPs have two alleles, which represent a substitution of one base for another. The SNP occurs at each allele of an individual may be different. If the SNP occurs more frequently in the general population, it is called “major” allele. In contrast, if the frequency of the SNP exist is rare in the population, it is designated the “minor” allele. Since human have two copies of chromosome or diploid, therefore, an individual can have various genotypes such as homozygous of major or minor alleles, or heterozygous of major and minor allele [41]. Many SNPs are correlated with one another, so it is difficult to distinguish the SNP that affects the phenotype from the several SNPs associated with it [44].

SNPs are identified and characterized by sequencing the same genomic region in several populations [45] [46]. The sample size of the population being resequenced is important. In general, larger sample sizes are needed to identify SNPs on the lower end of the minor allele frequency spectrum. The minor allele frequency (MAF) refers to the frequency at which the less common allele occurs in a given population. By using population genetics theory prediction for a SNP detection rate of 99%, a SNP with a minor allele frequency of 5% or greater needs 48

chromosomes, whereas a SNP with a minor allele frequency of 1% or greater requires 192 chromosomes for the verification of genotype of SNP [47]. An example of SNPs in miRNA-146a is the rs2910164 which is involved and associated in many diseases and it's prognosis [48].

Conclusion:-

We found dysregulation of miR-146 and its target genes were the main responsible for many autoimmune diseases. So further studies about single nucleotide polymorphisms concerning the mentioned miRNA is recommended.

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