THE ROLE OF miRNA IN AUTOIMMUNE DISEASES OF THE CONNECTIVE TISSUE

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S u m m a r y. Autoimmune diseases of the connective tissue are chronic, and are characterized by unclear etiology. Therefore, it is often difficult to choose a potential therapy. The research conducted over the last years into miRNA profiling, and the effects of this molecule on the pathophysiological processes typical of various autoimmune diseases has provided opportunities for a better understanding of the mechanism of these diseases and, in many cases, the introduction of new and more effective therapies.

K e y w o r d s: autoimmune disease, miRNA, rheumatoid arthritis, systemic scleroderma, Sjögren's syndrome

Autoimmune diseases

Autoimmune diseases are chronic conditions affecting 4 -7% population, more commonly females and the elderly. Genetic predisposition, immune, environmental and hormonal factors are thought to be involved in the pathogenesis of autoimmune diseases. They may be organ-specific (e.g. Addisson's disease, visceral disease, multiple sclerosis) or organ-non-specific (e.g. Sjögren's syndrome - SjS, systemic scleroderma - SSc, systemic lupus erythrematosus – SLE, Sjögren's syndrome, rheumatoid arthritis – RA) [63].

miRNA

MicroRNA (miRNA) is a small non-coding RNA single-stranded molecule of about 22 nucleotides in length. It is involved in the post-transcription regulation of many genes due to the complementarity of base pairs to messenger RNA (mRNA) [18, 33, 80]. Gene silencing is achieved via degradation of selected mRNA or inhibition of transcript translation [9]. miRNA involvement was demonstrated in the pathogenesis of various pathologies, congenital regulation, and acquired immune response [34, 56]. miRNA is one of the most abundant short regulatory RNA group [28].

The synthesis of miRNA includes several stages. In the transcription phase, primary transcripts are created (pri-microRNA). Later they are processed to form an approximately 70-nucleotide precursor microRNA (pre-microRNA). This precursor is subsequently transported to the cytoplasm where it is processed into mature miRNA of approximately 22 nucleotides [11].

miRNA molecule was first reported in 1990s. Investigating *Caenorhabditis elegans* the researchers found that short RNA molecules - let-7 and lin-4, later called miRNAs, were responsible for proper transition from the first to second larval stage and from the fourth stage to the mature stage of the nematode [8]. In 2006, Andrew Z. Fire and Craig C. Mello received the Nobel Prize in Biology and Medicine for discovering the RNA-iRNA interference phenomenon [67].

The role of miRNA in autoimmune diseases

Rheumatoid arthritis (RA)

Rheumatoid arthritis is an autoimmune systemic chronic disease affecting the connective tissue. RA affects app. 0.5-1% world population and

women are affected 2.5 times more frequently compared to men. It often develops in individuals with common variable immunode□ciency (CIVD) [37, 57, 69, 70].

The causes of RA have not been fully established. RA is thought to result from the immune imbalance due to the loss of galactose particles from IgG Fc fragments due to galactosylotransferase de□ciency in B lymphocytes [31].

RA is characterized by progressive inflammatory conditions of symmetrical joints occurring in the synovial membrane that leads to the destruction of articular and paraarticular tissues. As a consequence, the function of the articular system gets disrupted, which later results in disability or premature death due to increased risk of cardiovascular complications [10, 37]. RA usually affects proximal interphalangeal joints in the hands and feet, causing acute pain, reduced movement and swelling [70]. The inflammatory reaction within the synovium causes destruction of the articulating surfaces of the bone, distortion of the affected joints, and damage to the ligaments. That leads to the accumulation of immunocompetent cells involved in the immune response [70].

Immune disorders not only affect joints, but they also produce systemic manifestations involving peripheral blood mononuclear cells responsible for the secretion of abnormal pro- and anti-inflammatory cytokines.

Clinical course of RA is characterized by periods of exacerbation, with intermittent silencing of symptoms; in rare cases their complete remission is observed [32].

RA is diagnosed on the basis of criteria established by the American College of Rheumatology – ACR, and the European League Against Rheumatism – EULAR (2010) [1].

Currently, patients with inflammation not caused by other pathology of the synovial membrane within at least one joint are clinically assessed. De□nitive diagnosis of RA is made if the patient's score is at least 6 [64].

RA is treated by glycocorticosteroids and disease modifying drugs (DMDs). In RA exacerbation periods nonsteriodal anti-inflammatory drugs are used (NSAIDs), and if those are ineffective, anti-cytokine treatment with tumor necrosis factor alpha (TNF-α), and anti-CD20 or anti-CD80/86 antibodies are implemented [64, 77].

miRNA in rheumatoid arthritis

Recently, altered miRNA expression in RA patents has been observed by the researchers examining miRNA pro□le in various biological samples using an array of techniques (PCR, qRT-PCR, NGS, etc.). Researchers suggest essential involvement of miRNA in molecular mechanisms triggering the onset and progress of RA.

Nagata et al. observed decreased miRNA-15a expression (in mouse model) of RA responsible for the induction of cell apoptosis in the synovial membrane of mice with RA compared to controls [43]. Pauley et al. found increased miRNA-16 expression in mononuclear blood cells in RA patients compared to healthy individuals, and miRNA-16 expression depended on the disease activity, which was also con□rmed by Murata et al. [42, 48].

Moreover, plasma miRNA-24 expression was found to be increased in RA patients [24]. Another study observed decreased miRNA-124a expression in RA patients in comparison to patients suffering from osteoarthrosis (OA) [75].

The comparison of plasma miRNA-132 expression in RA or OA patients found that miRNA-132 was signi□cantly decreased in the affected patients compared to healthy controls. Moreover, it was found that miRNA-132 in the synovial fluid can be used as a diagnostic marker as it allows to differentiate between RA and OA [44]. The results contradict Pauley's □ndings of increased miRNA-132 expression determined by peripheral blood mononuclear cells count (PBMC) in RA patients compared to the control group [48].

Research into miRNA-146a found increased expression in RA patients compared to OA patients [45, 62, 79]. Quantitative analysis of miRNA-146a expression in the synovial fluid or T CD4+ lymphocytes isolated from the synovial membrane con□rmed suspected increased miRNA-146a expression in RA compared to OA, and its correlation with the disease activity [81, 34].

Moreover, increased miRNA-146aexpression in the plasma, peripheral blood mononuclear cells or T CD4+ lymphocytes was determined in RA patients compared to healthy subjects [42, 48]. miRNA-146a inhibits intracellular signaling pathway of nuclear NF-kB responsible for osteoclastogenesis control [45, 63, 81].

In another study, the researchers used peripheral blood mononuclear cells and observed miRNA-155 expression was 1.8 – 2.6 times higher in RA patients compared to the controls [42, 48]. Stańczyk

et al. found a signi□cant increase in miRNA-155 expression in the □broblasts isolated from the synovial membrane in the patients with RA in comparison to OA patients [45, 62, 79]. The investigation of miRNA-155 function revealed the involvement of that molecule in the control of metalloproteinases MMP-3 and MMP-1 expression [39, 45].

Alsaleh et al. observed direct influence of miRNA-346 on IL-18 release via inhibition of Bruton's tyrosine kinase expression [3]. Decreased expression of miR-363 and miR-498 in T CD4+ lymphocytes isolated from the synovial fluid was noted in the patients with RA compared to the healthy controls [34].

Systemic sclerosis (SSc)

Systemic sclerosis (SSc) is a rare chronic disease affecting the connective tissue [19, 86]. It is characterized by diffuse □brosis, degenerative □brosis in the tissues, complex immune disorders affecting various internal organs, and vasculopathy [5, 47, 50, 66, 86]. Speci□c clinical features of SSc include skin manifestations, which is one of basic diagnostic criterion (according to the classi□cation by the American College of Rheumatology – ACR [21, 50].

The disease produces manifestations in several body systems, e.g. circulatory, digestive, urinary, and the skeletal system where it is manifested as sclerodactyly [74]. In early-stage SSc joint deformities are observed in 12-65% patients, with the disease progression the symptoms appear with higher frequency (46-97%) [41]. Scleroderma can develop in any joint, especially in the hands, wrists and feet, and produces arthralgia and stiffness of the ligaments and resultant crepitation. X-rasy can visualize para-articular calci□ed deposits [29, 72, 73].

There are two clinical forms of SS, i.e. limited scleroderma (lSSc), and diffuse scleroderma (dSSc) [53]. The pathogenesis of systemic sclerosis is complex and remains incompletely understood. Involvement of infectious factors like CMV, HBV, *Helicobacter pylori*, parvovirus C19, and chemical agents, e.g. polyvinyl chloride - PVC, L-tryptophan, silicon are known to be involved in the development of the illness [7, 23]. Moreover, there are reports suggesting the contribution of vitamin D de□ciency leading to immune disorders observed in the course of SSc [6, 12, 71]. Researchers also found strong correlation between SSc and histocompatibility complex of human leukocyte antigen HLA class II on chromosome 6.

Research data suggest a signi□cant involvement of gene polymorphism of connective tissue growth factor (CTGF), □brillin-1, interferon regulatory factor-5 (IRF-5), interleukin 1a (IL-1a), tumor necrosis factor-alfa (TNF-α), transforming growth factor-a, (TGF-α), and monocyte chemoattractant protein-1 (MCP-1) [56]. In laboratory serology, antinuclear antibodies: anti-topoisomerase I (Scl-70, ATA) and anti-centromere antibodies (ACA) were determined by indirect immuno-fluorescence (ELISA) and immunoblotting in the serum of SSc [24].

miRNA in systemic sclerosis

Research into the role of miRNA in SSc has been conducted worldwide, and the results so far have shown the role of this molecule in the pathogenesis of the disease, as illustrated in the literature review below.

A team of Japanese researchers headed by Makino K. conducted a study in the group of 61 SSc patients and 20 healthy controls. Serum miR-142-3p was determined by real-time PCR. The results found that expression of miR-142-3p was signi□cantly elevated in the patients with systemic sclerosis in relation to the control group, and the level of expression correlated with severity of the disease [36].

Recent experiments, published in Biomed Pharmacother, show that the increase in miR-202-3p expression is characteristic of skin lesions in patients with SSc compared to healthy subjects. The results and analysis of miR-202-3p role in the human body indicate that it may stimulate tissue □brosis in SSc patients by inhibition of MMP1 expression [83].

The study of miRNA-155 expression demonstrated its increased levels in the skin tissue of patients with SSc compared to healthy subjects, and the level of expression showed a strong positive correlation with the degree of skin lesions resulting from the ongoing disease process. Qingran Yan et al. hypothesize that miRNA-155 silencing may inhibit collagen synthesis, and be one of the potential treatments for systemic sclerosis [82].

Journal of Clinical Immunology (2012) published a study on a group of 14 participants (7 patients with SSc and 7 healthy subjects). The examination of the □broblasts obtained from tissue cultures of biopsized samples revealed considerably increased levels of miRNA-21 expression in SSc patients, while miRNA-145 and miRNA-29b levels were decreased compared to the control group [84].

Honda et al. have shown that miRNA-150 may play a significant role in the pathogenesis of SSc as it influences over-expression of P3 integrin. The miRNA expression profile was determined by miRNA microarray PCR and real-time PCR. The level of fibroblast miR-150 expression was reduced in SSc patients, both *in vivo* and *in vitro,* compared to healthy subjects [27].

Sing et al. investigated serum and fibroblast miRNA-92a expression in 61 patients with systemic sclerosis. They found significantly higher levels of miRNA-92a expression in the patients with SSc compared to the healthy subjects, which may be due to internal TGF-β activation as a negative feedback mechanism towards integrin over-expression [60].

Sjögren's syndrome (SjS, SS)

Sjögren's syndrome (SjS, SS) is one of the most common autoimmune systemic diseases of the connective tissue [80]. The incidence of SjS in the general population ranges from 0.6 to 3-4%, however such large variation in epidemiological data is due to the type of diagnostic methods used, and the selection of the study group. Sjögren's syndrome is predominantly female, with the peak of incidence being between the age of 40 and 50 [51, 68].

In this disease, lymphoid cells infiltrate the exocrine glands and impair their function, which results in pathological changes in many systems and organs. Sjögren's syndrome mainly affects the salivary glands and lacrimal glands [20, 40, 76]. Other exocrine glands are rarely affected, and those are mainly mocus glands in the upper and lower airways, resulting in nasal, pharyngeal, and bronchial dryness. In the case of exocrine glands of the gastrointestinal tract, loss of gastric mucus lining and asymptomatic pancreatitis are observed. In some cases, SjS patients also report dyspareunia related to dryness in the external genitalia and the skin [17, 76]. The clinical symptoms include dry eye (*keratoconjunctivitis sicca*) experienced as sand under the eyelids, light sensitivity (photophobia), pain and burning or stinging of the eyes, and symptoms of dry mouth (xerostomy) which contributes to difficulty eating, swallowing, and speaking; it also leads to oral mucosa inflammations, tooth decay, and enlarged salivary glands [28, 40, 55].

The etiology of SjS is not fully understood. It is believed that genetic factors, hormonal disorders, especially insufficient secretion of sex hormones and resultant sexual dysfunction, viral infections, and psychoneuroimmunological factors play a significant role in the onset and progress of the disease [13].

SjS is classified as either primary or secondary. In primary SjS, histopathological picture of the affected organs is characterized by the presence of focal and diffuse cellular infiltrates, mainly T and B lymphocytes, which accounts for about 20-25% of the infiltration, and monocytes, macrophages, and natural killer cells (NK cells) responsible for less than 5% [67]. Primary Sjögren's syndrome occurs by itself and secondary Sjögren's syndrome occurs when another connective tissue disease is present. [35]. Secondary SjS is characterized by concomitant occurrence of other autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, thyroid disease, or systemic sclerosis [4, 8, 14, 15, 22, 49, 56].

Primary SjS is treated by relieving medications to ease the symptoms, i.e. saliva-stimulating preparations, artificial tears, and immunosuppressive synthetic drugs such as methotrexate (primarily prescribed in arthritis), cyclosporine, cyclophosphamide, or biological drugs like rituximab (anti-CD20) [80]. Treatment of secondary SjS is focused on the underlying disease and easing dryness [52]. Diagnosis is made on the basis of classification criteria developed by ACR in 2012 [59].

To determine SjS, blood tests can be done to confirm patient has high levels of antibodies that are indicative of the condition, such as antinuclear antibody (ANA), anti-Ro, anti-La antibodies, and hypergammaglobulinemia (80%), anemia, leukopenia, rheumatoid factor, and cryoglobulins [25].

miRNA in Sjögren's syndrome

Pauley et al. undertook research into the expression of a particular type of miRNA in Sjören's syndrome. They examined 25 patients with SjS and 10 healthy controls. They collected samples of the peripheral blood mononuclear cells (PBMCs), and found that the average relative level of miRNA-146a expression was 8-fold higher in SjS patients compared to the control group. MiRNA-155 expression was 2.5-fold higher in SjS patients compared to healthy subjects. No significant changes in miRNA-132 expression were observed that could provide a criterion to diagnose SjS patients.

miRNA-146a expression studies were also performed on a mouse model using PBMCs and the salivary glands. The experiments confirmed increased expression of this molecule in SjS animals

compared to the control group, both at the disease onset and at its advanced stage. Functional analysis showed that miRNA-146a contributes to increased phagocytosis and suppression of inflammatory cytokines and nitric oxide, which may indicate its signi□cant role in triggering or progression of Sjögren's syndrome [30].

The above quoted results also con□rm the results of the experiments carried out in Hungary by Erik Zilahi et al. They examined PBMCs collected from 21 patients diagnosed with SjS. The □ndings con□rmed increased miRNA-146a and miRNA-146b expression in the patients compared to healthy controls [85].

Huan Shi and co-workers examined 27 patients with SjS and 22 healthy individuals. They isolated PBMCs, and using real-time PCR and analyzing the symptoms found in the patients, they concluded that SjS patients had a signi□cant increase in miRNA-146a expression compared to healthy subjects, while miRNA-155 expression was signi□cantly lower compared to the control group. It should be noted that their results are contradictory to the results of Pauley's study [58].

Synopsis

In the future, modern therapy for patients with autoimmune diseases of the connective tissue may be based either on the induction or inhibition of various miRNA molecules expression. Recent advances in molecular biology and genetics related to microRNA pro□ling and the effects of this molecule on the pathophysiological processes characteristic of various types of autoimmune diseases will provide a better understanding of the mechanism of these diseases, and in many cases, will help understand the failure of existing treatments. This will improve the ability to diagnose, rheumatoid arthritis, systemic sclerosis, and Sjögren's syndrome at an early stage. Furthermore, more effective prognosis and implementation of treatment will probably be reflected in reduced mortality, and will offer a better quality of life for patients.

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