

# REVIEWS

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## Plasmatic microRNA as Potential Biomarkers of Multiple Sclerosis: Literature Review\*

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### Abstract

There is ongoing research with the goal of finding precise and sensitive biomarkers of multiple sclerosis (MS). Recently, researchers have paid particular attention to small, non-encoding, single stranded endogenous microRNA molecules (miR, miRNA). At first these molecules were thought to be found only within the cell. Today it is known, however, that they can also be found in the extracellular spaces (plasma, serum, saliva, urine, tears, sweat, milk, sperm and amniotic fluid, among others). It has been established that extracellular miRNA perform a wide spectrum of functions, such as transmitting signals between cells, modulating processes involved in angiogenesis, neurogenesis, proliferation or apoptosis. Given the high stability of these small molecules in the extracellular compartment (plasma), their tissue specificity and strong ties with pathological processes underlying multiple sclerosis, miRNA seem to be a good target for researchers trying to discover diseases' new markers. Determining an accurate miRNA expression profile in MS and correlating it with the gene profile may lead to the discovery of new pathophysiological processes. Demonstrating that changes in the composition and concentration of extracellular miRNA may in some cases correlate with certain aspects of the underlying disease (such as its severity) could lead to their use as biomarkers of MS. Further research is needed (*Adv Clin Exp Med* 2016, 25, 4, 775–779).

**Key words:** multiple sclerosis, plasma miRNA, biomarkers, role of miRNA in MS.

There is ongoing research with the goal of finding precise and sensitive biomarkers of multiple sclerosis (MS). Recently, researchers have paid particular attention to small, non-encoding, single stranded endogenous microRNA molecules (miR, miRNA). At first these molecules were thought to be found only within the cell. Today it is known, however, that they can also be found in the extracellular spaces (plasma, serum, saliva, urine, tears, sweat, milk, sperm and amniotic fluid, among others). It has been established that extracellular miRNA perform a wide spectrum of functions, such as transmitting signals between cells, modulating processes involved in angiogenesis, neurogenesis, prolifera-

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\* This article was based on a doctoral thesis (in Polish) written by one of the authors, entitled "Evaluation of microRNA expression as potential biomarkers of activation of the immune system in the course of Multiple Sclerosis (MS)" and contains excerpts thereof.

## MicroRNA (miRNA, miR): The Best Understood Class of Short, Non-Regulatory RNA

First described in 2006, there is a ubiquitous expression of single stranded, endogenous, conservative and noncoding RNA molecules (ncRNA), between 19 and 25 nucleotides in length, in most living organisms. At the time little was known about their function in the cell. Today it is known that miRNA can be differentiated by their expression profile and nucleotide sequence [1, 2]. They also play a crucial role in the posttranscriptional regulation of gene expression. Sequences encoding regulatory miRNA molecules can be found in the human genome within genes encoding protein (both in introns and exons), intragenic regions or as independent genes encoding solely RNA with no protein product. It is currently believed that up to 30% of human genes are regulated by miRNA [1, 2]. The biogenesis of miRNA is composed of a number of steps that begin in the nucleus and end in the cytoplasm [3, 4]. First of all, a part of the genome is transcribed by RNA polymerase II, forming the so-called primary RNA transcript (pri-miRNA). Those primary transcripts now have to undergo a series of changes brought about by various enzymes in a process called 'miRNA maturation' [1]. Still in the nucleus, the pri-miRNA are cleaved by the Drosha enzyme into shorter intermediate forms – the miRNA precursors (pre-miRNA). The precursors are between 60 and 80 nucleotides long, and have a stem loop (hairpin) appearance [3, 4]. Drosha is a type-III RNase which cuts through the base of the stem and thus generates a double stranded RNA with a phosphate group at the 5'-end and a hydroxyl group and two unpaired nucleotides at the 3'-end, typical of this class of enzymes. The now freed miRNA precursors are subsequently transported from the nucleus to the cytoplasm by means of Exportin-5 (Exp5) transporter proteins working together with a Ran-GTP protein [4]. In the cytoplasm, before mature and functional miRNA molecules can be formed, the precursors have to be subjected to the actions of several proteins, such as the Dicer endonuclease, Argonaut protein (AGO) or transactivating response RNA-binding protein (TRBP) [2–4]. Mature miRNA molecules – unwound by the Dicer enzyme – are coupled with several proteins to form a ribonucleoprotein complex called RISC (RNA-induced silencing complex). RISC can form com-

plementary bonds with the target mRNA, thereby influencing its stability and translation. Mature miRNA are made up of two-stranded RNA. One of the strands (known as the guide strand) is incorporated into the RISC, while the other strand (known as the passenger strand, or miRNA\*) is usually degraded (new research suggests, however, that it may also take part in gene silencing) [5].

## Characteristics of Extracellular miRNA

Although most miRNA can be found within the cell, many can also be found in the extracellular compartment, where they end up as a result of cellular death (apoptosis or necrosis) or injury. Extracellular miRNA are not merely the remnants of dead cells. Instead, their excretion and preservation are actively controlled by means of cellular signaling [6, 7]. Examples include active excretion in the form of microvesicles (exosomes) and apoptotic bodies, binding into complexes by special proteins, such as Ago (an RNase inhibitor) or active transport by the HDL particles [8]. The presence of miRNA within microvesicles circulating in peripheral blood under physiological conditions suggests they play a role in intracellular communication and regulation of gene expression [9, 10].

Research has demonstrated that microvesicles isolated from human plasma originate mainly in platelets, mononuclear phagocytes and, to a lesser degree, T lymphocytes and endothelial cells. It is possible that the miRNA contained in microvesicles are transported to specific tissues. The microvesicle selection process would likely be regulated by the so-called P-bodies found in the proximity of miRNA in the cytoplasm [9]. Based on the assumption that the transfer of miRNA *via* exosomes is an active process, one may surmise that such a process must be regulated by either protein found within the vesicle itself or special receptors found on target cells, or both [9]. Recent studies show that miRNA molecules transported between cells inside of exosomes are indeed biologically active [9]. Extracellular miRNA functions are varied, and include relaying signals between cells and modulating angiogenesis, neurogenesis, cell proliferation and apoptosis, among others [10]. Changes in composition and concentration of miRNA in the extracellular compartment are in many cases related to pathological processes ongoing in the body, which may allow them to be used in some cases as biological markers of disease activity [11].

## **Plasma miRNA as Biomarkers of Multiple Sclerosis**

### **Multiple Sclerosis**

MS is a lifelong autoimmune inflammatory disorder of the central nervous system, involving a number of pathological processes such as the infiltration of lymphocytes and macrophages, degeneration of axons and neurons, local demyelination, remyelination or astrogliosis, among others. The disease usually manifests itself in young adults between 20 and 40–45 years old [12]. Some of the most commonly cited factors involved in the pathogenesis of MS are environmental factors (understood as climate, diet, certain pathogens or toxins), infections, genetic predisposition and autoimmunity. The geographical distribution of MS correlates with latitude and vitamin D3 deficiencies. The search for more precise biomarkers of MS is still underway [12].

### **The Stability of Plasma miRNA and Their Use as Biomarkers**

Ideally a biochemical marker of MS should be highly specific and measurable in blood or plasma (Biomarkers Definition Working Group). Furthermore, its blood concentration should correlate with its content in the cerebrospinal fluid (CSF) [13]. Such a marker should appear in various bodily fluids shortly after the onset of the disease, and should react in response to treatment in order to be used as a prognostic tool. Blood plasma is a rich source of specific miRNA, which could potentially be useful in diagnosing neoplasias, cardiovascular diseases and brain injuries as well as MS, as they had been found to correlate with disease progression, activity, prognosis and response to treatment [14, 15]. In the existing literature, cell-free circulating miRNA are rarely the main focus, as it has been believed to be degraded by ribonucleases. The molecules, however, proved to be very stable and resistant to the process of endogenous RNases present in blood [16]. Different miRNA expression profiles between tissues could suggest that miRNA perform different biological functions in different tissues [17, 18]. Discovering a characteristic expression profile could be of key importance in the early diagnosis of MS [14, 19–21]. Several researchers have similarly tried to correlate the expression of certain miRNA with patients' general condition measured by the EDSS score and disease duration in patients with relapsing-remitting and secondary-progressive disease courses [19, 20].

## **The Role of Plasma miRNA in the Activation of the Immune System in Multiple Sclerosis**

Despite knowledge of over 100 kinds of miRNA [22, 23] involved in regulating gene expression in immune system cells, analyses to-date have not yielded a marker with high enough specificity and sensitivity to be of use in the diagnosis of MS. MicroRNA in the immune system function mainly by regulating transcription factors, proapoptotic protein and almost every element of the signal transduction cascade. Proteins regulated by miRNA are critical in many cellular processes, and even small changes in their levels lead to notable results. It has been demonstrated that one miRNA molecule can control several different mRNA, while one mRNA can contain binding sites for several different miRNA [23]. miRNA regulating the development and differentiation of immune cells shows different levels of expression in the thymus and bone marrow. Notable examples of molecules involved in T- and B-cell development include miR-150, miR-180 and the miR17~92 cluster [23, 24]. Stimulating the miR-17~92 cluster was proven to increase levels of activated B-cells, CD4+ T-cells, and to a lesser degree CD8+ T-cells, which in turn contributed to the development of autoimmune disorders. Some miRNA are also strong active regulators of monocyte differentiation. MiRNA may be treated as an intermediate factor between innate and adaptive immune responses. A mounting nonspecific immune response often leads to abrupt changes in miRNA expression levels. The search continues for new precise plasmatic biomarkers of the activation of the immune system in the course of MS.

## **The Role of Plasma miRNA in the Development and Course of Inflammation in Multiple Sclerosis**

Certain miRNA are involved in mounting an acute inflammatory reaction by regulating the production of antibodies and the release of the mediators of inflammation [25, 26]. Inflammatory response can most likely be influenced either way, by upregulating either the pro- or anti-inflammatory cytokines [27]. One of the better understood regulatory molecules is miR-16, seen in high concentrations in cells involved in inflamma-

tion (monocytes, neutrophils, B-cells, CD4+ and CD8 T-cells). Its action is due to swift degradation of the mediators of inflammation, such as IL-6 and TNF-alpha [27]. Another molecule of interest is miR520/373, inhibiting the signaling pathways induced by NF- $\kappa$ B. By acting directly on the gene encoding NF- $\kappa$ B (the *RELA* gene), it reduces the expression of proinflammatory cytokines IL-6 and IL-8 [27, 28]. miR-146 and miR-155, on the other hand, are molecules with strong proinflammatory properties, created as a result of the activation of toll-like 4 (TLR4) receptor's signaling pathway [29]. miR-126 regulates levels of the VCAM-1 adhesion molecule on endothelial cells, while miR-31, miR-17-3b and miR-221 affect levels of selectin E and ICAM-1 molecules [29–32]. To this day, the scientific understanding of the development and course of the inflammatory response in MS is limited at best. Further research is essential, and the role of plasma miRNA should not be overlooked.

### Selection of miRNA as Precise Biomarkers of Multiple Sclerosis

Finding precise and sensitive biomarkers of MS, as described by the Biomarkers Definition Working Group, has been a goal of many scientists for a considerable amount of time. Such tests would prove very useful in diagnosing patients with dubious MRI results. A potential biomarker should be easy to detect, and as such, those molecules whose concentrations go up in readily available bodily fluids are better candidates. In the case

of blood and plasma, the biological material is plentiful and allows for more diagnostic and scientific testing. Current knowledge of the biological functions of extracellular miRNA is still very limited. Early studies have indicated that cellular miRNA and extracellular miRNA found in plasma could be negatively correlated [13, 19, 30]. MicroRNA seem to be universal prognostic factors. Interestingly, expression profiles of miRNA can be specific for a particular clinical disease course of MS.

### Conclusions

Extracellular miRNA have a wide variety of functions. Their high stability in bodily fluids, tissue specificity, and close ties to mechanisms involved in the pathogenesis of MS make these molecules promising targets for further research. The diagnosis of MS is based on documenting changes in the brain and the spinal cord, disseminated in space and time. In cases when a clinical diagnosis poses difficulties due to mixed symptomatology, neuroimaging and biomarker detection in the CSF or other body fluids is of increased importance. In addition to being an aid in diagnosis, markers could help in prognosing outcomes, assessing response to treatment or even estimating susceptibility to the disease in healthy patients. Even now, the role of small, non-coding miRNA as biomarkers is still not clear. Papers published on the subject have thus far all been carried out on small groups of patients. It is safe to assume, however, that in years to come, many more studies focusing on this topic will take place.

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