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Editorial

MicroRNAs as promising novel biomarkers and potential drug targets for inflammatory neurological diseases

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This issue of the Journal of the Neurological Sciences features a paper by Rostedt Punga and co-workers who determined the serum levels of two microRNAs (miRNAs), selected based on their initial profiling study [1], in patients with Myasthenia gravis (MG). They describe significantly elevated levels of miR-150 and miR-21 in serum of MG patients compared both to healthy blood donors and several autoimmune disease controls (Psoriasis, Addison's and Crohn's disease). Furthermore, the levels of these miRNAs were reduced in MG patients on immunosuppressive treatment compared to MG patients without such treatment.

MG represents a prototypical organ specific autoimmune disease, where an autoreactive humoral response is directed against the postsynaptic endplate of the neuromuscular junction, and alike many other autoimmune conditions it features a high degree of complexity in terms of aetiopathogenesis, prognosis and therapeutic response [2]. Apart from clinical features, such as generalized or ocular symptoms and the possible presence of thymic pathology, stratification of MG patients today is largely limited to determination of autoantibodies directed towards the skeletal postsynaptic muscle proteins, mainly the acetylcholine receptor (AChR) and the muscle-specific receptor tyrosine kinase (MUSK). There are indications that subgroups defined by autoantibody status respond differently to treatment, but it is of limited clinical value at the individual level to follow titers over time. A large number of studies in MG have examined immune related biomarkers such as cytokines and chemokines, recently reviewed in [3]. However, this type of protein-based biomarkers have important limitations relating to technical aspects, such as sensitivity to degradation and need for less exact immune-based measurement protocols, but also to what degree a pattern of dysregulation is specific for a particular disease.

In the last decade the biomarker field has undergone a dramatic evolution, with the discovery of several new classes of biological compounds or chemical modifications that both can be measured and shed light on relevant disease mechanisms. Although more work is needed, this progress also fuels hopes for clinically valuable tools for diagnosis, prediction of prognosis and identification of responders to therapy or individuals at risk for certain side effects. Non-coding RNAs (ncRNAs), which do not have protein-coding potential, have recently emerged as putative biomarkers for human diseases.

Two decades after their initial discovery, miRNAs are today recognized as important regulators of most biological functions and comprise the most abundant class of gene regulatory molecules. According to miRBase (release 21, 2014), 35 828 mature miRNAs have been annotated in 223 species (http://www.mirbase.org/). Mature miRNAs are small, around 22 nucleotides (nt), evolutionary conserved endogenous ncRNAs that mediate post-transcriptional regulation of gene expression (reviewed by [4]). It has been estimated that a single miRNA has the potential to regulate hundreds of target genes and that >60% of human protein-coding genes is under miRNA control.

Most miRNAs are transcribed by Polymerase II into long primary miRNA transcripts (pri-miRNAs) that are processed in the nucleus by the Drosha–DGCR8 complex to approximately 70 nt precursor hairpin structures (pre-miRNAs). After nuclear processing, the pre-miRNAs are exported to the cytoplasm by Exportin-5 coupled with Ran-GTP where they are cleaved by the Dicer–TRBP complex to approximately 22 nt duplex miRNA molecules. Duplexes are incorporated into an Argonaute protein-containing miRNA-induced silencing complex (RISC), followed by retention of the mature miRNA strand in RISC complex where the miRNA targets complementary sites of the targets mRNAs. Biogenesis of miRNA is essential for viability and knockout mice lacking Dicer die early in development.

The interaction between miRNA and mRNA in the RISC complex is mediated by perfect base pairing of the miRNA "seed" region (nucleotides 2–8 in the mature miRNA) with complementary nucleotides in the 3′ untranslated region (UTR) of the target mRNA [5]. This binding generally results in a reduction of protein product, predominantly due to mRNA decay but also through translational repression.

Since miRNAs play important regulatory roles in nearly all biological processes it is not surprising that they are frequently found dysregulated in a variety of human diseases. Indeed, miRNAs have been implicated among others in cancer, infection, cardiovascular, inflammatory and autoimmune, neurodegenerative and metabolic diseases. This has prompted recent efforts to develop miRNA-based therapeutic interventions. Current strategies rely on either restoring the function of a miRNA (by miRNA mimics) or inhibiting the function of a miRNA (by antagomiRs or sponges) (reviewed by [6]). Particularly appealing is the fact that miRNAs target multiple targets and thus therapeutics based on targeting a single miRNA have the potential to affect the entire pathway regulated by this miRNA. Additionally, by targeting the "seed" region it is possible to target the entire miRNA family that controls an overlapping set of targets with augmented beneficial effect. The first miRNA-based inhibitory drug that advanced to clinical trials is miravirsen (Santaris Pharma), which is an antagomiR that inhibits

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miR-122, thereby reducing hepatitis C virus (HCV) replication in the liver. In a clinical phase II trial miravirsen displayed dose-dependent and long-lasting antiviral activity in patients with chronic HCV infection [7]. The first miRNA-based replacement drug that entered clinic is MRX34 (Mirna Therapeutics), which is miR-34 mimic used in inoperable primary liver cancer [8]. Besides therapies targeting miR-122 and miR-34 a number of additional miRNA-based therapeutics are undergoing extensive preclinical testing.

With the advent of technologies for the global high-throughput screening of transcriptome, miRNAs have emerged as a new class of biomarkers. Given the heterogeneity of complex diseases it is likely that a panel of biomarkers is necessary to reflect different disease pathologies and miRNAs can fulfill that criterion. Associations between miRNAs, either in tissues or cells, have been investigated in nearly all diseases and conditions and many miRNAs have been suggested as potential biomarkers. For example, a classification system based on 48 miRNAs was suggested to accurately predict tissue origin in histologically confirmed metastatic cancer with unknown primary site of disease [9].

Following the discovery of the export of miRNAs into the extracellular space in 2007 and their detection in all bio-fluids, not only blood but also easy to collect urine and saliva, circulating miRNAs received rapid attention as promising non-invasive biomarkers. They are either packaged into membrane-bound vesicles such as exosomes or associated with protein complexes such as Argonaute 2 or with HDL particles. Circulating miRNAs are attractive due to their remarkable stability under different physiological and storage conditions [10]. Additionally, they display limited daily fluctuations in healthy subjects [11]. Last but not the least, multiple miRNAs can be quantified from small amount of material using specific and sensitive assays often based on quantitative real-time PCR.

Studies of miRNAs in MG are in their infancy with the first reports starting to emerge in 2012. It is inevitable that this field will suffer a similar destiny as miRNA-research in other complex diseases, including initial limited overlap between miRNAs implicated in different studies owing to small cohorts, heterogeneity of disease, differences in tissues and methodologies used. In that respect, the study by Rostedt Punga and co-workers, with so far the largest cohort, well-established methodology and building on preliminary findings, represent a significant step forward. Future studies are warranted to demonstrate the true potential of miR-150 and miR-21, alone or in combination with other miRNAs, to act as biomarkers in clinical settings. However, miR-150 and miR-21 already now point to potentially interesting pathogenic mechanisms involving maturation, differentiation and activation of T cells (reviewed in [12]) that might open new avenues for immunomodulatory treatments in MG.

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