MicroRNAs (miRNAs) constitute a potent layer of gene regulation that has only been recognized since the early 2000s. MiRNA genes are encoded in the genomes of all higher eukaryotes and transcription of these genes gives rise to miRNA precursor molecules (figure). Such precursors are processed to 20–24 nucleotide single-stranded mature miRNAs, which are subsequently incorporated into the RNA-protein complex known as the RNA-induced silencing complex (RISC). Within RISC, the miRNA functions as guide and directs RISC to target sites located on mRNAs, which very often are only partially complementary to the miRNA. The physical association with RISC leads to a reduced protein production from the target mRNA (figure). About 1,000 human miRNAs have been identified so far. Since miRNAs are only partially complementary to target sites, potential targets for any given miRNA can be predicted for many different miRNAs. It is thereby hypothesized that miRNAs regulate the expression of almost half of all human genes. Consequently, dysregulation of miRNAs has been linked to inflammatory, degenerative, and neoplastic neurologic diseases. Furthermore, miRNAs can leave their intracellular environment where they normally function, and enter the bloodstream and other body fluids. The function of such circulating miRNAs remains largely elusive, but recent evidence indicates a possible role of miRNAs in cell-to-cell communication.

In this issue of Neurology®, Haghikia et al. present the first analysis of miRNAs in the CSF of patients with multiple sclerosis (MS), with intriguing results. First they performed a general miRNA profiling of the cell-free CSF of pooled MS vs pooled control CSF, analyzing 760 miRNAs with an array. Based on expression level and differential abundance, they selected 5 miRNAs for a subsequent study, using a commercially available qPCR approach allowing for the selective quantification of different miRNA species. The second study was performed with 53 patients with MS and 39 controls, including patients with other inflammatory CNS diseases. They confirmed that 2 miRNAs were higher in patients with MS, while one was reduced in MS. Two of these miRNAs differentiated secondary progressive MS (SPMS) from relapsing-remitting MS (RRMS). Three features seem important. First, this difference was also seen when patients with MS were compared with patients having other inflammatory diseases. Second, levels of these miRNAs were not correlated with the CSF cell number, suggesting against the idea that observed increases of miRNAs are based on disintegration of CSF cells before the cell-free supernatant has been obtained. Third is the exciting difference between SPMS and RRMS.

Previous studies had suggested a role of miRNAs in the pathogenesis of MS based on the identification of dysregulated miRNAs in MS lesions and on altered miRNA profiles in blood cells (reviewed in references 6 and 7). The analysis of miRNAs in MS tissue lesions is greatly facilitated by the fact that miRNAs can be readily quantified from archival formalin-fixed and paraffin-embedded tissue using qPCR, even from those specimens that do not allow an mRNA quantification. This is possible because of the short length of miRNAs and their embedding in intracellular RISCs.

While most of the studies about miRNA involvement in diseases were based on the analysis of tissue specimens or blood cells, the presence of miRNAs in body fluids has been appreciated just recently. The stability of miRNAs in serum—and also in the spinal fluid, as reported here—makes them attractive candidates for biomarker analysis. miRNAs appear to be incredibly stable in serum; they are either packaged into secreted vesicles or incorporated into RISCs. In both cases, miRNAs are protected from serum nucleases. In a recent study, it was demonstrated that cells may communicate with each other via secreted miRNAs: miRNAs were secreted from endothelial cells by packaging into extracellular vesicles, taken up by smooth muscle cells, and the transferred miRNAs could indeed regulate gene expression in the recipient cells. However, it is still elusive how pervasive such effects of circulating miRNAs are, and future functional studies will be required to unravel the underlying mechanisms.

The pioneering work by Haghikia et al. encourages future studies about miRNAs in the CSF, not only in patients with MS (box). Additional important issues to be

See page XXX
addressed include whether miRNAs in body fluids are actively secreted, or found as a consequence of dying cells, and whether there are specific miRNA signatures in the CSF correlated with certain inflammatory disease courses, or even forms of cancer that metastasize into the CNS.

**miRNAs in the cell-free CSF**

**Current state:**
1. MiRNAs present in the CSF are identified; they are highly stable and can be quantified
2. Evidence for alterations in MS vs other neurologic diseases and other inflammatory neurologic diseases, even RRMS vs SPMS

**Future issues**
1. Establish the best set of miRNAs used for testing of cell-free CSF
2. Distinct profiles in different CNS inflammations: MS, neuromyelitis optica, neuroborreliosis, limbic encephalitis?
3. Transition from RRMS to SPMS associated with altered miRNAs in the CSF? Predictive value of miRNA profiles in patients with CIS for MS conversion?
4. Effect of therapy on miRNA profiles in the CSF?
5. Source of miRNAs in CSF?
6. Function beyond biomarker? Communication between cells via secreted miRNAs?

**DISCLOSURE**

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**REFERENCES**
