into two different subsets MO and PMN-MDSCs, based on the expression of Ly6C and Ly6G, very important in infectious, autoimmune and tumor models. The present work will further characterize the potential role of miR-223 in the EAE model and MS. First we found an upregulation of miR-233 in the Peripheral Blood Mononuclear Cell (PBMC) of 20 MS samples vs. 20 controls (fold change over controls 1.64 ± 1.25 vs. 1.20 ± 0.95, $P = 0.018$). This result was confirmed in a different cohort of subjects, including 15 untreated MS subjects (population from Italy: 11 RRMS, 4 PPMS) and 12 healthy controls. In this cohort, miR-233 was upregulated in MS vs. control subjects (fold change over controls 0.81 ± 0.65 vs. 0.40 ± 0.26, $P = 0.010$). We also performed several active EAE experiments in miR-223 knockout (miR-223 KO) mice and littermate control mice. MiR-223 KO mice developed a significantly less severe disease ($P < 0.0001$ by two-way ANOVA) with a significantly higher percentage of PMN-MDSC (CD11b/Ly6G positive cells) and MO-MDSC (CD11b/Ly6C positive cells) in the spleens and spinal cords compared to control mice. We found also that MO-MDSC from miR-223 KO mice had greater immune-suppressive effects on $CD4^+$ T cell proliferation than controls in antigen T cell stimulatory conditions. It is established that MO-MDSCs inhibit $CD4^+$ and $CD8^+$ T cell proliferation mostly via ARG1 action. ARG1 was promptly upregulated in MO-MDSC from miR-223 KO cells corresponding to their high immunosuppressive function. These results demonstrate altered levels of miR 223 in the PBMC of MS patients and suggest that miR-223 plays a role in EAE. This may lead to the identification of new disease biomarkers of therapeutic targets.

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Methylome characterization of $CD4^+$ T cells in multiple sclerosis — Establishing a role for miR-21 in autoimmune disease

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Evidence for methylation changes in $CD4^+$ T cells and brain tissue of multiple sclerosis (MS) patients and healthy controls (HC) strongly suggests a role for epigenetics in disease pathogenesis. We here sought to identify methylation changes in one of the key players in MS disease, $CD4^+$ T cells, between MS patients and HC. The $CD4^+$ T cells were sorted using a MoFlow sorter from peripheral blood mononuclear cells (PBMCs) isolated from MS cases and HC. DNA extracted from the $CD4^+$ T cells was subjected to genome-wide DNA methylation quantification using Illumina Infinium Human Methylation 450K Bead chip. The top scoring changes in DNA methylation between groups were confirmed using bisulfite pyrosequencing and miRNA expression was detected by TaqMan microRNA-assay. Preliminary analyses of the genome-wide methylation data revealed higher methylation rate at all CpG positions of the miR-21 gene in MS patients compared to controls. In line with this finding there was a lower miR-21 expression in these patients as methylation is generally associated with gene silencing. We further investigated the involvement of miR-21 in miR-$21^{-/-}$ and wild type mice in an animal model of MS, experimental autoimmune encephalomyelitis (EAE). miR-$21^{-/-}$ mice were protected against EAE as compared to littermate controls. Taken together these findings support the notion that epigenetic regulation of miR-21 expression affects autoimmune neuroinflammation. To mechanistically dissect the impact of miR-21 on EAE, the inflammatory response will be characterized in miR-$21^{-/-}$ animals and littermate controls during the course of EAE.

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In situ microRNA profiles of astrocytes in the context of ischemic brain injury and multiple sclerosis

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Anatomic distribution is an important factor that impacts on the functional properties of astrocytes under pathologic conditions. MicroRNAs (miRNAs) are small ribose nucleic acid (RNA) molecules that function as post-transcriptional regulators of gene expression. Distinct miRNA profiles have been associated with pro-inflammatory and anti-inflammatory responses. In this study, we analysed inflammation-related miRNA expression profiles of human glial fibrillary acidic protein (GFAP)-immunoreactive cells laser-capture microdissected from brain samples of adults with multiple sclerosis (MS) and ischemic infarcts or from control brains (with no specific pathology). When comparing miRNA profiles of astrocytes isolated from grey and white matter regions of control cases, we found differences in relative expression of specific miRNAs that have previously been detected in human astrocytes in vitro: MiRNA-125b and miRNA-338 were upregulated in white matter astrocytes; while miRNA-145 and miRNA-181a were upregulated in grey matter astrocytes. No differences in expression of the known proinflammatory miR-155 or the anti-inflammatory miR-146a were seen between white matter and grey matter astrocytes of these control cases. In cases of ischemic lesions, astrocyte expression of miR-155 was similar to the expression observed in control cases, whereas miR-146a expression was downregulated in both grey and white matter astrocytes of the ischemic lesions. Similarly, miR-155 expression in astrocytes captured from active MS lesions did not differ from that from controls, while their expression of miR-146a was downregulated. Our study demonstrates grey versus white matter regional differences in miRNA profiles of human astrocytes under non-pathologic conditions and selective abnormalities in the regulation of individual miRNAs involved in regulating inflammatory responses under pathological conditions.

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Differentially expressed microRNAs in multiple sclerosis patients alter regulatory T cells

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Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system, which results in a wide range