Identification of microRNAs in the cerebrospinal fluid as marker for primary diffuse large B-cell lymphoma of the central nervous system

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The diagnosis of primary central nervous system lymphoma (PCNSL) depends on histopathology of brain biopsies, because disease markers in the cerebrospinal fluid (CSF) with sufficient diagnostic accuracy are not available yet. MicroRNAs (miRNAs) are regulatory RNA molecules that are deregulated in many disease types, including cancer. Recently, miRNAs have shown promise as markers for cancer diagnosis. In this study, we demonstrate that miRNAs are present in the CSF of patients with PCNSL. With a candidate approach and miRNA quantification by reverse transcription polymerase chain reaction, miRNAs with significant levels in the CSF of patients with PCNSL were identified. *MiR-21*, *miR-19*, and *miR-92a* levels in CSF collected from patients with PCNSL and from controls with inflammatory CNS disorders and other neurologic disorders indicated a significant diagnostic value of this method. Receiver-operating characteristic analyses showed area under the curves of 0.94, 0.98, and 0.97, respectively, for *miR-21*, *miR-19*, and *miR-92a* CSF levels in discriminating PCNSL from controls. More importantly, combined miRNA analyses resulted in an increased diagnostic accuracy with 95.7% sensitivity and 96.7% specificity. We also demonstrated a remarkable stability of miRNAs in the CSF. In conclusion, CSF miRNAs are potentially useful tools as novel noninvasive biomarker for the diagnosis of PCNSL. (*Blood*. 2011;117(11):3140-3146)

Introduction

Unraveling the cause of focal brain lesions in patients with unexplained neurologic symptoms remains a clinical challenge. Especially in patients with primary central nervous system lymphoma (PCNSL), definitive diagnosis often is not possible on the basis of radiographic features and responsiveness to corticosteroids, which both do not specifically distinguish between lymphoma and inflammatory central nervous system (CNS) disease.[1,2] In most patients with suspected CNS lymphoma who present with rapidly deteriorating neurologic symptoms, stereotactic brain biopsy remains the diagnostic procedure of choice. However, CNS biopsies are associated with the risk of hemorrhage and neurologic damage, and a definitive histopathologic diagnosis cannot always be achieved.[1]

Because PCNSLs represent highly aggressive tumors, early diagnosis is essential for successful treatment and improvement of disease prognosis.[1,3-5] Although evaluation of the cerebrospinal fluid (CSF) is less invasive than brain biopsy, cytopathologic, immunophenotypic, and genetic analyses of CSF cells are much less sensitive.[6-8] Protein markers within the CSF include antithrombin,[9] soluble CD27,[10] and free immunoglobulin light chains.[11] They have been shown to be helpful with improved diagnostic sensitivities; however, their utility in accurate diagnosis of PCNSL from the CSF has not finally been established.[1]

MicroRNAs (miRNAs) are small regulatory RNA molecules that bind the 3′-untranslated regions of mRNA transcripts and inhibit gene expression at a posttranscriptional level by interference with translational initiation or degradation of mRNA.[12,13] In several studies, miRNAs have been shown to play key regulatory roles in a wide range of genetic pathways that control cellular differentiation, proliferation, and apoptosis in physiologic conditions as well as different human diseases. There is increasing evidence that dysfunctional expression of miRNAs is a common feature of many types of cancer, and miRNAs play a direct role in cancer because they can function as oncogenes and tumor suppressors.[13] Deregulated miRNA expression is found in various malignancies, including leukemia and lymphoma.[12]

MiRNAs are increasingly used as markers for diagnostic and prognostic purposes.[14] Expression analyses of miRNAs can be accomplished directly from tumor samples. Furthermore, circulating miRNAs stably packaged in microvesicles can be detected in human serum and plasma.[14] Previously, it has been shown that high expression levels of distinctive miRNAs are detectable in sera from patients with different types of cancers and are related to disease prognosis, for example, B-cell lymphoma,[15] prostate cancer,[16] and non-small cell lung cancer.[17] Until today, there is no report of miRNAs identified in the CSF of patients with primary diffuse large B-cell lymphomas of the CNS.

In this study, we hypothesized that miRNAs could be useful CSF-based markers for detection of PCNSL. A candidate miRNA approach assessing miRNA expression by quantitative reverse

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