Published in final edited form as:

J Cell Physiol. 2013 May ; 228(5): 1070–1075. doi:10.1002/jcp.24254.

Cerebrospinal Fluid miRNA Profile in HIV-Encephalitis†

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Abstract

MicroRNAs are short non-coding RNAs that modulate gene expression by translational repression. Because of their high stability in intracellular as well as extracellular environments, miRNAs have recently emerged as important biomarkers in several human diseases. However, they have not been tested in the cerebrospinal fluid (CSF) of HIV-1 positive individuals. Here, we present results of a study aimed at determining the feasibility of detecting miRNAs in the CSF of HIV-infected individuals with and without encephalitis (HIVE). We also evaluated similarities and differences between CSF and brain tissue miRNAs in the same clinical setting. We utilized a high throughput approach of miRNA detection arrays and identified differentially expressed miRNAs in the frontal cortex of three cases each of HIV+, HIVE, and HIV– controls, and CSF of ten HIV-positive and ten HIV-negative individuals. For the CSF samples, the group of HIV+ individuals contained nine cases of HIV-Associated Neurological Disorders (HAND) and, among those, four had HIVE. All the HIV-negative samples had non-viral acute disseminate encephalomyelitis. A total of 66 miRNAs were found differentially regulated in HIV+ compared to HIV– groups. The greatest difference in miRNA expression was observed when four cases of HIVE were compared to five non-HIVE cases, previously normalized with the HIV-negative group. After statistical analyses, eleven miRNAs were found significantly up-regulated in HIVE. Although more clinical samples should be examined, this work represents the first report of CSF miRNAs in HIV-infection and offers the basis for future investigation.

Keywords

miRNA; HIV; CSF

†This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/jcp.24254]

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The Authors declare no conflict of interests.

Introduction

HIV-associated neurological disorders (HAND) comprise cognitive, motor and behavioral impairments which affect a substantial number of HIV-1 infected individuals. While the introduction of the highly active antiretroviral therapy (HAART) has greatly reduced the incidence of HIV-associated dementia, the prevalence of HIV-associated minor cognitive and motor disorders has increased (reviewed in (Gannon et al., 2011)). Understanding the biology of HIV-1 infection in the brain and identifying markers for its pathological manifestations, including HIV-encephalitis, are of critical importance. Although several molecules in the CSF have been identified as putative indicators of neurocognitive impairments, the need for biomarkers persists (McGuire, 2009).

After over a decade of investigation of miRNAs, it is now clear that these non-coding RNA molecules serve a fundamental role in the regulation of gene expression; even though specific regulation and function of miRNAs are largely unknown. Similarly to mRNAs, some miRNAs show specific tissue distribution, such as miR-124 which is enriched in the central nervous system (CNS) (Mishima et al., 2007). Changes in the expression of cellular miRNAs have been associated with a variety of pathologic conditions (Montano, 2011), including neurodegenerative disorders (Eacker et al., 2009; Sonntag, 2010), and systematic high throughput approaches, such as miRNA profiling arrays, have lead to the identification of miRNA signatures in several types of cancer (Erkan et al., 2011; Gibcus et al., 2009; O'Hara et al., 2008; Pedranzini et al., 2010; Robertus et al., 2010; Shah et al., 2010). Finally, emerging evidence accumulated supporting the concept of establishing microRNAs expression profiles in body fluids as possible diagnostic and prognostic markers (Alevizos and Illei, 2010; Cho, 2010; De Smaele et al., 2010; Ferracin et al., 2010; Heneghan et al., 2010; Ju, 2010; Kosaka et al., 2010; Taft et al., 2009; Wittmann and Jack, 2010; Xie et al., 2010). Although the literature confirms a growing interest for miRNAs and their potential role in HIV (Eletto et al., 2008; Noorbakhsh et al., 2010; Rom et al., 2010; Tatro et al., 2010; Yelamanchili et al., 2010), to our knowledge this is the first report of CSF miRNA profile.

We have utilized RT-PCR-based miRNA arrays to detect expression of 742 unique miRNAs in the CSF of HIV-1-positive individuals with HAND compared to HIV-1-negative individuals. After normalization, 66 miRNAs were considered for further analysis. Comparison between HIV-1 and HIV+ (non-HIV) groups showed 47 up- and 7 down-regulated miRNAs in HIV-1. Statistical analysis (ANOVA followed by Dunnet's test) indicated significant differences ($p < 0.05$) among the three groups for 11 miRNAs, with miR-182*, miR-362-5p, miR-720, and miR-937 having a p -value < 0.05 . Using a similar approach we have also obtained miRNA profiles from the frontal cortex of HIV+, HIV-, and HIV- clinical samples. Although the CSF specimens and frontal cortices were obtained from different individuals, our results show a surprisingly high number of miRNAs deregulated in both types of specimens.

Altogether, our study demonstrates feasibility in detecting CSF miRNAs, it provides a putative CSF miRNA signature for HIV-1, and shows important correlation in differentially regulated miRNAs in the frontal cortex and CSF samples.

Materials and Methods

Clinical samples

CSF samples were collected over a period of five years from patients at the University of Milan and stored frozen at -80°C in aliquots until analysis. Formalin-fixed paraffin

embedded (FFPE) samples from the frontal cortex were previously obtained from the Manhattan HIV Brain Bank (Dr. Susan Morgello, Mt. Sinai, New York).

RNA preparation and miRNA array

RNA was extracted from 0.5 ml of CSF using the mirVANA miRNA isolation kit (Ambion, Austin, TX) following the manufacturer's instructions. Carrier RNA (MS2 RNA, Roche) was added at 1 µg per sample prior to total RNA extraction (Andreasen et al., 2010). RNA was quantified using the NanoDrop spectrophotometer and 40 ng were retrotranscribed using the universal cDNA synthesis kit (Exiqon, Woburn, MA). Three RT reactions were performed for each sample. Each cDNA reaction was mixed with SYBR Green master mix (Exiqon) and dispensed into two 384 well plates containing 742 unique miRNAs and several controls (Exiqon microRNA ready to use PCR, human panels I and II). PCR was performed with a Roche LightCycler 480 System (Roche Applied Science, Indianapolis, IN) following Exiqon's recommended settings.

For the FFPE samples, RNA was extracted using the high pure miRNA isolation kit (Roche) from two 10 µm thick sections, 1 cm × 1 cm each.

Data processing and statistical analyses

Absolute quantification using 2nd derivative maximum was calculated with Roche Lightcycler 480 software. Exiqon arrays were performed in triplicate and qPCR data were analyzed in GenEx Professional 5 software (MultiD Analyses AB, Goteborg, Sweden). If a miRNA was not present in at least two out of three replicas it was set as non-expressed. Because of the low abundance of miRNAs in the CSF, we set a cut off of 39. If a miRNA had a cycle threshold Ct >39 for one probe, that reading was replaced with the average of the Ct for the other two probes, provided they were both below 39. Relative quantification was determined by the ΔCt method using miR-622 and miR-1266 as reference genes, as determined by GeNorm and Normfinder incorporated in the GenEx software. The control HIV-negative group was used as the calibrator between the HIVE and non-HIVE groups and the fold change was calculated using the formula $2^{-\Delta\Delta\text{Ct}}$. miRNAs with a fold change <0.5 were considered down-regulated, and miRNAs with a fold change >2 were set as up-regulated. The three groups, HIV-, HIVE, and HIV+ for the CSF experiment were compared using oneway ANOVA allowing for unequal variances, and followed by Dunnet tests. For this exploratory study a cut off for the *p*-value was set at 0.2.

For the analysis of brain tissue miRNAs, miR-650 and miR-1266 were used as reference genes. Due to the low number of samples analyzed (*n*=3 for each group), statistical analysis was not performed on the experiments related to the brain tissue samples.

Bioinformatics

Predicted gene targets for the eleven differentially regulated miRNAs were identified using miRWalk module (Dweep et al.), which allows the search of up to eight established target prediction programs. The list of genes predicted by three out of five databases was filtered for the brain tissue using DAVID functional annotation bioinformatics microarray analysis (Huang da et al., 2009a; Huang da et al., 2009b). Next, Gene Ontology (GO) annotations of predicted gene targets were determined using Genego integrated software (MetaCore).

Results

RNA was extracted from a total of 20 CSF samples, 10 HIV-negative and 10 HIV-positive, and subjected to miRNA arrays. The group of HIV-positive individuals consisted of nine cases of HAND and was further divided into HIV-encephalitis (HIVE) (*n*=4) and non-HIVE

($n=5$) cases. The HIV-negative group comprised all cases of acute disseminated encephalomyelitis of non-viral origin and none of the individuals in this group had cognitive impairments. Males and females were represented in both HIV⁻ and HIV⁺ groups. The age of the subjects ranged from 36 to 66 years old, with an overall average of 52.7 (51.3 for the HIV⁺ and 54.1 for the HIV⁻ group). Each sample was retrotranscribed in triplicates and the resulting cDNA was loaded into three sets of miRNA plates. The Exiqon miRNA ready plates contain preassigned 6 wells for 6 different genes which have stable expression levels over a wide range of sample types. Three of these are microRNAs which are often stably expressed, and the other three are small RNA reference genes. However, using Normfinder and GeNorm applications incorporated in the GEnEx analysis software, we found that in CSF the most uniformly expressed miRNAs were miR-622 and miR-1266; therefore, we have selected them as reference genes. The CSF samples were divided in three groups, HIV⁻ ($n=10$), HIV⁺ ($n=5$) and HIVE ($n=4$) and the ratio HIVE/HIV⁺ was determined after normalization of HIVE and HIV⁺ with the control HIV⁻ group. For comparison analyses, only miRNAs present in all the three groups were selected and a new list of 66 miRNAs was generated (Figure 1); while miRNAs expressed preferentially in only one or two groups were removed. In general, infection with HIV-1 results in an overall down-regulation of miRNAs (Figure 1, lane HIV⁺/HIV⁻; values < 0.5 highlighted in green), with only five up-regulated miRs (highlighted in red). In contrast, in the group of cases with encephalitis (lane indicated with HIVE/HIV⁻; values between 0.5 and 2) the majority of miRNAs showed unchanged expression levels and only three, miR-937, miR-598, and miR-595 were up-regulated. Finally, an opposite trend in miRNA expression was observed when HIVE and HIV⁺ groups were compared and most of miRNAs showed up-regulation, while only seven were down-regulated (Figure 1, lane indicated with HIVE/HIV⁺). Expression of 11 miRNAs, miR-502-5p, miR-877*, miR-127-5p, miR-543, miR-1247, miR-615-3p, miR-574-3p, miR-663, miR-197, miR-485-3p, and miR-766 remained unchanged across the three groups of samples (Figure 1, highlighted in yellow). The list of 66 miRNAs was further subjected to ANOVA analysis followed by the Dunnet's test to determine statistically significant differences among the three groups. Eleven miRNAs, miR-1203, miR-1224-3p, miR-182*, miR-19b-2*, miR-204, miR-362-5p, miR-484, miR-720, miR-744*, miR-934, and miR-937 were found differentially expressed with a $p < 0.2$ (Figure 2). Within this group, miR-182*, miR-362-5p, miR-720, and miR-937 had the lowest p -value, $p < 0.05$.

We also evaluated miRNA profiles in archived brain tissues previously obtained from the Manhattan HIV Brain Bank. We analyzed three cases each of the following: HIV⁻ control, HIV⁺, and HIVE. Comparison between groups was performed as described for the CSF study in which the fraction HIVE/HIV⁺ represents the ratio of the fold changes between the two groups HIVE and HIV⁺, calculated using the HIV⁻ group as control. The results show 121 differentially regulated miRNAs (Figure 3). In general, and similar to the CSF miRNA profiles, HIV-infection resulted in overall down-regulation of miRNAs. In contrast, in the presence of encephalitis miRNA levels increased. This trend is clear when we compare HIVE versus HIV⁺ (ratio HIVE/HIV⁺) in which most of the miRNAs were up-regulated (Figures 1 and 3).

Next, we compared CSF and frontal cortex miRNA profiles and the results are shown in Figure 4A. Thirty five of the 66 CSF miRNAs (53%) were also present in the list of differentially regulated miRNAs in the frontal cortex. Four miRNAs, miR-1296, miR-134, miR-16, and miR-495 had comparable expression changes for the ratio HIVE/HIV⁺ in both CSF and brain tissue, with miR-1296 being the most up-regulated. Differences in the expression level of six out of the 11 statistically significant CSF miRNAs were plotted in Figure 4B. Strikingly, miR-937 is more than 20 times up-regulated in the CSF compared to frontal cortex in HIVE cases.

Next, we investigated predicted gene targets for the 11 statistically significant miRNAs (Figure 2) in the context of maps, networks and diseases and the results are shown in Figure 5. The most represented genes appear to be related to the maps of cytoskeleton remodeling, cell adhesion and chemokines (Figure 5, upper table). Various aspects of neurogenesis including axonal guidance, notch signaling, synaptogenesis and transmission of nerve impulse were the most represented networks (Figure 5, middle table). Interestingly, the predicted gene targets for the combined miRNAs were associated with mental disorders such as mood, affective and depressive disorders, as highlighted in Figure 5 (lowest table).

In summary, our findings indicate an overall down-regulation of miRNAs in HIV-infected individuals in both CSF and frontal cortex. This trend, however, appears to be reversed in the presence of encephalitis, with miR-937 being the most up-regulated. While more clinical samples should be profiled to validate a miRNA signature in the CSF of individuals with HIV-1, with and without encephalitis, this work provides knowledge and important procedural aspects to investigate CSF miRNAs in the clinical setting of HIV-infection.

Discussion

HIV enters the CNS early during infection, frequently leading to cognitive impairments that can persist even after successful viral suppression (Heaton et al., 2010; Power et al., 2009; Woods et al., 2009). HIV-associated neurological disorders comprise a variety of cognitive and motor impairments that range from mild to severe and that are characterized by molecular events leading to neuronal injury, inflammation, and loss of neuroprotection. While several indicators of neuronal injury and immune activation have been identified in the CSF, they do not correlate with the severity and/or progression of HAND, and the need of diagnostic and/or prognostic markers is high priority (McGuire, 2009). MicroRNAs have emerged as critical regulators of gene expression in mammals. Several features of miRNAs, including their stability and tissue specificity, make them suitable for their detection and relative quantification in a variety of tissues and body fluids. In contrast to most mRNAs, detection of miRNAs in specimens such as formalin-fixed tissue and cerebrospinal fluid (CSF) or other body fluids is more accurate and reliable (Doleshal et al., 2008; Mitchell et al., 2008). However, there are some limitations that have been outlined in a recent review by Pritchard et al. (Pritchard et al., 2010). Accordingly, we found that recovery of the RNA for qPCR miRNA profiling was higher in the brain samples (FFPE) in comparison to CSF specimens. Differently from chip-based microarrays, which generally use micrograms of RNA, the advantage of using quantitative PCR panels is that they require nanogram amounts of RNA, allowing diagnostic analyses from low RNA recovery type of tissues, such as CSF and FFPE samples. Furthermore, the low RNA abundance could be, at least partially, overcome by pre-amplification cycles, for which kits have been recently developed and are commercially available. Although the global normalization approach is widely used in microarrays (Pradervand et al., 2009; Wylie et al., 2011), it couldn't be applied to our system due to the low number of miRNAs that were represented in all samples (Mestdagh et al., 2009). Eight miRNAs had an average Ct < 37 and two of them, miR-622 and miR-1266, that showed uniform expression across all samples were chosen as reference genes. Of interest, expression levels of miR-1266 remained unchanged across the samples and in the brain tissue. MiR-937 was the most abundant miR in all CSF samples with an average Ct of 33.6, while in the frontal cortex miR-720 (average Ct=23) was the most expressed miRNA. Although the number of the analyzed brain samples was lower than CSF cases, we have observed significantly less variability across the samples in the brain tissues compared to the CSF. Despite of this difference in RNA samples a high number of miRNAs were found differentially regulated in both brain and CSF. Accordingly, 53% of differentially regulated miRNAs in the CSF were also present in the frontal cortex (Figure 4A).

Figure 1 shows a global downregulation of miRNA expression in the CSF of HIV+ individuals compared to HIV- controls (Figure 1, lane HIV+/HIV-). This trend is reversed in the presence of encephalitis (compare lanes identified as HIVE/HIV- and HIV+/HIV-). A similar general miRNA down-regulation was also observed in the frontal cortex as shown in Figure 3. Overall, these data may suggest impairment in the processing of miRNAs (synthesis and/or maturation). Indeed, we have some indication from gene expression arrays performed on the same brain tissue cases that levels of proteins involved in the miRNA biogenesis, such as Dicer and DCGR8, are down-regulated (data not shown). Among the most up-regulated miRNAs in the CSF of HIVE cases, miR-19b-2*, miR-937, and miR-362-5p had the largest fold change. Although only 11 CSF miRNAs showed statistically significant differences, several other miRNAs may be promising in that they show a large fold change, such as the up-regulated miR-595, miR-300, miR-598, miR-1296, miR-555, miR-222, and miR-105*, or the down-regulated miR-92b* and let-7d*.

Gene ontology analysis performed on the predicted gene targets for the eleven differentially regulated miRNAs revealed maps of genes associated with neurogenesis, cytoskeletal remodeling, chemokine signaling, and networks related to synaptogenesis and axonal guidance (Figure 5). Overall, bioinformatics analysis of CSF miRNA predicted gene targets may further provide additional information on the molecular events that lead to neuronal damage and/or persistent inflammation.

In summary, this is the first report detecting CSF miRNA in HIV patients and identifying a potential miRNA signature for HIVE. By demonstrating the possibility of detecting miRNAs in the CSF, this work offers both scientific and technical details for future longitudinal studies aimed to determine miRNA profiles in clinical settings that include antiretroviral therapies and/or addictive behaviors, such as alcohol and drug usage, often associated with HIV-infection.

Acknowledgments

This work was supported by the NIH grant MH 079751 and by the Stanley S Scott Cancer Center of the Louisiana Cancer Research Consortium (LCRC) to FP, and P60 AA009803-18 to SN.

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miRNA	HIVE/HIV-	HIV+/HIV-	HIVE/HIV+	miRNA	HIVE/HIV-	HIV+/HIV-	HIVE/HIV+
hsa-miR-19b-2*	1.89	0.04	44.18	hsa-miR-502-5p	1.69	1.07	1.58
hsa-miR-595	2.18	0.08	25.72	hsa-miR-877*	1.48	1.19	1.24
hsa-miR-300	1.86	0.09	19.90	hsa-miR-127-5p	1.34	1.14	1.18
hsa-miR-937	4.51	0.27	16.78	hsa-miR-543	1.16	1.16	1.00
hsa-miR-598	2.41	0.17	14.50	hsa-miR-766	1.15	1.03	1.11
hsa-miR-1296	1.47	0.12	12.46	hsa-miR-1247	1.08	1.03	1.05
hsa-miR-362-5p	1.09	0.09	12.15	hsa-miR-615-3p	1.04	0.61	1.71
hsa-miR-555	1.17	0.13	9.25	hsa-miR-574-3p	1.02	1.06	0.96
hsa-miR-222	1.34	0.16	8.30	hsa-miR-663	1.00	0.63	1.59
hsa-miR-105*	1.48	0.20	7.57	hsa-miR-197	0.70	0.93	0.75
hsa-miR-708*	1.20	0.19	6.32	hsa-miR-485-3p	0.69	0.76	0.91
hsa-miR-520h	0.87	0.14	6.22				
hsa-miR-1471	0.91	0.15	6.11				
hsa-miR-452	1.52	0.25	5.99				
hsa-miR-19a	1.31	0.22	5.96				
hsa-miR-650	1.45	0.25	5.71				
hsa-miR-629	1.36	0.24	5.63				
hsa-miR-137	0.66	0.12	5.46				
hsa-miR-562	0.92	0.17	5.32				
hsa-miR-29b-1*	1.33	0.27	4.85				
hsa-miR-212	0.78	0.17	4.71				
hsa-miR-520d-3p	0.86	0.19	4.55				
hsa-miR-141*	1.61	0.37	4.38				
hsa-miR-888	1.12	0.27	4.13				
hsa-miR-139-5p	1.02	0.25	4.10				
hsa-miR-16	1.15	0.32	3.58				
hsa-miR-558	1.25	0.35	3.55				
hsa-miR-346	1.32	0.38	3.50				
hsa-miR-518f*	0.68	0.20	3.45				
hsa-let-7c	1.60	0.48	3.33				
hsa-miR-134	1.38	0.42	3.25				
hsa-miR-182*	1.68	0.53	3.19				
hsa-miR-720	0.89	0.28	3.15				
hsa-miR-1224-3p	1.48	0.48	3.07				
hsa-miR-302c*	1.08	0.35	3.05				
hsa-miR-297	1.02	0.34	3.04				
hsa-miR-1203	1.35	0.45	3.00				
hsa-miR-744*	1.60	0.53	2.99				
hsa-miR-524-5p	1.12	0.39	2.86				
hsa-miR-934	1.45	0.54	2.69				
hsa-miR-1539	1.70	0.65	2.62				
hsa-miR-1913	0.64	0.26	2.46				
hsa-miR-490-3p	1.53	0.62	2.45				
hsa-miR-495	1.06	0.44	2.43				
hsa-miR-204	1.19	0.53	2.22				
hsa-miR-1269	1.12	0.51	2.18				
hsa-miR-885-3p	0.98	0.45	2.18				
hsa-miR-450b-3p	0.54	0.40	1.35				
hsa-miR-760	0.88	1.86	0.48				
hsa-miR-324-3p	1.00	2.25	0.45				
hsa-miR-331-5p	0.64	1.43	0.45				
hsa-miR-187	1.06	2.45	0.43				
hsa-miR-484	0.78	3.01	0.26				
hsa-miR-92b*	1.28	7.49	0.17				
hsa-let-7d*	0.56	10.80	0.05				

Figure 1.

Differentially regulated CSF miRNAs. MiRNAs were profiled in ten HIV+ and ten HIV- CSF samples. Nine cases in the HIV+ group had HAND and, among those, 4 had HIVE. After data processing, comparison between three groups, HIVE, HIV+ and HIV- was performed as follows. First the HIVE and HIV+ groups were normalized for the HIV- control group according to the $\Delta\Delta Ct$ formula and then the fold change was calculated as the ratio HIVE/HIV+. Over-expressed miRNAs (fold change > 2) are indicated in red, under-expressed miRNAs in green (values < 0.5), and miRNAs whose expression was unchanged (values between 0.5 and 2.0) are highlighted in yellow.

miRNA	pvalues		
	Overall	HIV+	HIVE
hsa-miR-1203	0.137	0.458	0.504
hsa-miR-1224-3p	0.112	0.790	0.199
hsa-miR-182*	0.035	0.590	0.170
hsa-miR-19b-2*	0.145	0.221	0.716
hsa-miR-204	0.182	0.188	0.845
hsa-miR-362-5p	0.011	0.038	0.946
hsa-miR-484	0.167	0.374	0.386
hsa-miR-720	0.029	0.023	0.950
hsa-miR-744*	0.179	0.170	0.404
hsa-miR-934	0.167	0.953	0.133
hsa-miR-937	0.044	0.932	0.042

Figure 2.

List of miRNAs with differential expression significant at the 0.2 level. Global column *p*-values are for overall tests on the 3 groups (HIVE, HIV+ and HIV-). Dunnet tests comparing each of HIVE and HIV+ to HIV- were carried out. A total of 66 miRNAs was subjected to these analyses.

miRNA	HIVE/HIV-	HIV+/HIV-	HIVE/HIV+	miRNA	HIVE/HIV-	HIV+/HIV-	HIVE/HIV+
hsa-let-7b	0.138	0.004	38.408	hsa-miR-204	0.003	0.016	5.296
hsa-miR-145	0.161	0.004	36.758	hsa-miR-151-5p	0.013	0.067	5.260
hsa-miR-331-3p	0.641	0.022	29.651	hsa-miR-186	0.026	0.132	5.081
hsa-miR-221	0.740	0.032	23.480	hsa-miR-106a	0.013	0.065	5.063
hsa-miR-138	0.816	0.036	22.864	hsa-miR-29a*	0.124	0.613	4.925
hsa-miR-103	0.381	0.018	21.706	hsa-miR-27b	0.021	0.103	4.925
hsa-miR-30b	0.160	0.008	20.846	hsa-miR-33a	0.160	0.785	4.908
hsa-miR-885-5p	0.166	0.008	20.749	hsa-miR-324-3p	0.030	0.147	4.862
hsa-miR-129-5p	1.280	0.063	20.393	hsa-miR-99b	0.017	0.083	4.801
hsa-miR-127-3p	0.273	0.014	19.585	hsa-let-7d*	0.049	0.233	4.719
hsa-miR-30c	0.104	0.005	19.472	hsa-miR-27a	0.047	0.220	4.697
hsa-miR-149	0.324	0.020	16.393	hsa-miR-766	0.140	0.599	4.272
hsa-miR-132	0.329	0.020	16.280	hsa-miR-193b	0.024	0.099	4.108
hsa-miR-135a	0.228	0.014	16.111	hsa-miR-197	0.015	0.061	4.005
hsa-miR-21	0.147	0.010	15.419	hsa-miR-598	0.158	0.632	3.991
hsa-miR-1296	0.416	0.028	14.757	hsa-miR-222	0.027	0.107	3.991
hsa-miR-142-3p	0.771	0.054	14.156	hsa-miR-16	0.011	0.042	3.936
hsa-miR-29c	0.087	0.006	14.042	hsa-miR-140-3p	0.023	0.087	3.771
hsa-let-7f	0.094	0.007	13.269	hsa-miR-23b	0.028	0.105	3.694
hsa-miR-342-3p	0.116	0.009	12.996	hsa-miR-664	0.021	0.073	3.422
hsa-miR-143	0.097	0.008	12.743	hsa-miR-124	0.013	0.044	3.356
hsa-miR-99a	0.153	0.012	12.395	hsa-miR-191	0.018	0.059	3.272
hsa-miR-26a	0.072	0.006	11.713	hsa-miR-320a	0.006	0.019	3.175
hsa-let-7a	0.086	0.007	11.592	hsa-miR-885-3p	0.042	0.134	3.167
hsa-miR-139-5p	0.644	0.057	11.392	hsa-miR-125a-5p	0.008	0.023	3.109
hsa-miR-29a	0.096	0.008	11.379	hsa-miR-34a	0.028	0.088	3.095
hsa-miR-181b	0.121	0.011	11.197	hsa-miR-629	0.290	0.848	2.928
hsa-miR-328	0.264	0.026	10.291	hsa-miR-877*	0.423	1.227	2.901
hsa-miR-101	0.168	0.017	9.883	hsa-miR-584	0.093	0.251	2.704
hsa-miR-107	0.387	0.040	9.747	hsa-miR-134	0.039	0.100	2.603
hsa-miR-92b	0.123	0.013	9.747	hsa-miR-9	0.012	0.031	2.597
hsa-miR-1913	0.174	0.018	9.736	hsa-miR-25	0.014	0.037	2.588
hsa-miR-136	0.529	0.056	9.448	hsa-miR-495	0.074	0.187	2.523
hsa-miR-128	0.152	0.017	9.021	hsa-miR-593	0.141	0.338	2.403
hsa-miR-126*	0.073	0.008	9.000	hsa-miR-622	3.157	7.456	2.362
hsa-let-7c	0.127	0.014	8.990	hsa-miR-452	0.579	1.312	2.266
hsa-miR-24	0.151	0.017	8.674	hsa-miR-2110	0.066	0.142	2.171
hsa-let-7g	0.204	0.024	8.535	hsa-miR-451	0.014	0.031	2.158
hsa-miR-181a	0.106	0.012	8.505	hsa-miR-331-5p	2.855	5.439	1.905
hsa-miR-423-3p	0.103	0.012	8.388	hsa-miR-934	0.361	0.676	1.875
hsa-miR-150	0.069	0.008	8.340	hsa-miR-346	0.053	0.098	1.866
hsa-miR-23a	0.052	0.006	8.311	hsa-miR-574-3p	0.065	0.115	1.765
hsa-miR-497	0.200	0.024	8.225	hsa-miR-605	0.019	0.034	1.757
hsa-miR-19b	0.091	0.011	8.196	hsa-miR-484	0.031	0.051	1.666
hsa-miR-29b	0.130	0.016	8.196	hsa-miR-125b	0.010	0.013	1.298
hsa-miR-1247	0.549	0.073	7.516	hsa-miR-558	0.390	0.493	1.266
hsa-miR-638	0.177	0.024	7.353	hsa-miR-720	0.037	0.045	1.211
hsa-miR-26b	0.112	0.015	7.235	hsa-miR-141*	1.539	1.815	1.180
hsa-let-7e	0.098	0.014	7.177	hsa-miR-615-3p	0.310	0.360	1.159
hsa-miR-886-3p	0.113	0.016	6.940	hsa-miR-338-3p	0.042	0.047	1.122
hsa-miR-218	0.595	0.087	6.845	hsa-miR-940	0.021	0.023	1.115
hsa-miR-320b	0.046	0.007	6.751	hsa-miR-663	0.067	0.072	1.064
hsa-miR-15a	0.110	0.017	6.394	hsa-miR-93	0.041	0.043	1.042
hsa-miR-32	0.141	0.022	6.364	hsa-miR-562	0.679	0.565	0.832
hsa-miR-212	0.582	0.092	6.298	hsa-miR-411*	0.522	0.421	0.808
hsa-miR-1224-3p	0.185	0.029	6.284	hsa-miR-937	2.570	2.012	0.783
hsa-miR-551a	0.202	0.035	5.856	hsa-miR-490-3p	0.599	0.422	0.704
hsa-miR-485-3p	0.265	0.046	5.762	hsa-miR-224*	0.405	0.270	0.667
hsa-miR-92a	0.086	0.015	5.736	hsa-miR-219-5p	0.042	0.027	0.642
hsa-miR-1260	0.070	0.013	5.464	hsa-miR-760	0.310	0.161	0.520
hsa-miR-324-5p	0.161	0.029	5.464				

Figure 3.

List of differentially regulated miRNAs in archived brain tissue samples. Three cases each of HIVE, HIV+, and HIV– were analyzed and results show 121 differentially expressed miRNAs. Up-regulated and unchanged miRNAs are indicated in red and yellow, respectively. Note that in the lanes indicated as HIV+/HIV– and HIVE/HIV– miRNAs are all down-regulated.

A

miRNA	HIVE/HIV-	HIV+/HIV-	Frontal Cortex	miRNA	HIVE/HIV-	HIV+/HIV-	CSF
hsa-let-7c	0.127	0.014	8.990	hsa-let-7c	1.60	0.48	-3.33
hsa-let-7d*	0.233	0.049	4.719	hsa-let-7d*	0.56	10.80	-19.34
hsa-miR-1224-3p	0.185	0.029	6.284	hsa-miR-1224-3p	1.48	0.48	3.97
hsa-miR-1247	0.549	0.073	7.516	hsa-miR-1247	1.08	1.03	1.05
hsa-miR-139-5p	0.644	0.057	11.392	hsa-miR-139-5p	1.02	0.25	4.10
hsa-miR-141*	1.815	1.539	1.180	hsa-miR-141*	1.61	0.37	4.38
hsa-miR-1913	0.174	0.018	9.736	hsa-miR-1913	0.64	0.26	2.46
hsa-miR-204	0.016	0.003	5.296	hsa-miR-204	1.19	0.53	2.22
hsa-miR-212	0.582	0.092	6.298	hsa-miR-212	0.78	0.17	4.71
hsa-miR-222	0.107	0.027	3.991	hsa-miR-222	1.34	0.16	8.30
hsa-miR-324-3p	0.147	0.030	4.862	hsa-miR-324-3p	1.00	2.25	-2.24
hsa-miR-331-5p	5.439	2.855	1.905	hsa-miR-331-5p	0.64	1.43	-2.24
hsa-miR-346	0.098	0.053	1.866	hsa-miR-346	1.32	0.38	3.50
hsa-miR-452	1.312	0.579	2.268	hsa-miR-452	1.52	0.25	5.99
hsa-miR-484	0.051	0.031	1.666	hsa-miR-484	0.78	3.01	-3.89
hsa-miR-485-3p	0.265	0.046	5.762	hsa-miR-485-3p	0.69	0.76	0.91
hsa-miR-490-3p	0.422	0.599	0.704	hsa-miR-490-3p	1.53	0.62	2.45
hsa-miR-558	0.493	0.390	1.266	hsa-miR-558	1.25	0.35	3.55
hsa-miR-562	0.565	0.679	0.832	hsa-miR-562	0.92	0.17	5.32
hsa-miR-598	0.632	0.158	3.991	hsa-miR-598	2.41	0.17	14.50
hsa-miR-615-3p	0.360	0.310	1.159	hsa-miR-615-3p	1.04	0.61	1.71
hsa-miR-629	0.848	0.290	2.928	hsa-miR-629	1.36	0.24	5.63
hsa-miR-720	0.045	0.037	1.211	hsa-miR-720	0.89	0.28	3.15
hsa-miR-766	0.599	0.140	4.272	hsa-miR-766	1.15	1.03	1.11
hsa-miR-877*	1.227	0.423	2.901	hsa-miR-877*	1.48	1.19	1.24
hsa-miR-885-3p	0.134	0.042	3.162	hsa-miR-885-3p	0.98	0.45	2.16
hsa-miR-934	0.676	0.361	1.875	hsa-miR-934	1.45	0.54	2.69
hsa-miR-937	2.012	2.570	0.783	hsa-miR-937	4.51	0.27	16.78

B **Figure 4.**

Comparison of CSF and brain tissue miRNA expression profiles. A) 35 of the 66 differentially expressed CSF miRNAs are also deregulated in the frontal cortex and are listed in alphabetical order. Values are expressed as indicated in Figure 1. Note that, for simplicity, although not in green, most of the values in column 1 (HIVE/HIV-) are down-regulated. B) Six of the 11 statistically significant CSF miRNAs were also present in the frontal cortex and their relative expression is shown in panel B.

Maps	pValue	Ratio	
Cytoskeleton remodeling_TGF, WNT and cytoskeletal remodeling	1.998E-13	59	111
Cytoskeleton remodeling_FAK signaling	4.381E-10	34	57
Cell adhesion_Chemokines and adhesion	3.318E-09	48	100
Development_GM-CSF signaling	3.865E-09	30	50
Cytoskeleton remodeling_Cytoskeleton remodeling	7.526E-09	48	102
Chemotaxis_CXCR4 signaling pathway	9.547E-09	23	34
Development_Flt3 signaling	1.178E-08	27	44
Transport_Clathrin-coated vesicle cycle	1.297E-08	37	71
Development_Hedgehog signaling	4.516E-08	27	46
Development_FGFR signaling pathway	4.601E-08	30	54

Networks	pValue	Ratio	
Development_Neurogenesis_Axonal guidance	7.476E-10	129	230
Signal transduction_NOTCH signaling	1.374E-09	131	236
Development_Neurogenesis_Synaptogenesis	1.556E-09	105	180
Development_Neurogenesis in general	2.676E-09	110	192
Neurophysiological process_Transmission of nerve impulse	1.661E-08	117	212
Development_Hedgehog signaling	6.337E-08	134	254
Signal transduction_WNT signaling	1.987E-07	98	177
Cell adhesion_Attractive and repulsive receptors	2.163E-06	94	175
Cytoskeleton_Regulation of cytoskeleton rearrangement	3.245E-06	97	183
Cell adhesion_Amyloid proteins	1.501E-05	100	195

Diseases	pValue	Ratio	
Psychiatry and Psychology	8.113E-20	1261	2837
Mental Disorders	1.233E-19	1252	2817
Pathological Conditions, Signs and Symptoms	2.804E-15	1755	4182
Pathologic Processes	1.726E-12	1157	2694
Mood Disorders	1.008E-11	536	1156
Affective Disorders, Psychotic	7.619E-11	339	695
Bipolar Disorder	8.230E-11	338	693
Depressive Disorder, Major	2.609E-10	440	942
Depressive Disorder	9.021E-10	440	949
Suicide	1.458E-09	84	134

Figure 5.

Annotation results from Gene Ontology analysis. Predicted gene targets for the eleven differentially regulated CSF miRNAs (miR-1203, miR-1224-3p, miR-182*, miR-19b-2*, miR-204, miR-362-5p, miR-484, miR-720, miR-744*, miR-934, and miR-937) were subjected to GO analysis. The three tables show the list of the most significant maps, networks and diseases, respectively.