



MicroRNA profiling in plasma of HIV-1 infected patients: potential markers of infection and immune status

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Background: Circulating miRNAs are recently used as promising biomarkers for infectious diseases. The aim of this study was to identify plasma miRNAs for infection detection and monitoring the immune status of HIV infection.

Methods: A cohort of 128 plasma samples from HIV-1 infected subjects and 37 samples from healthy donors were analyzed by TaqMan Low-Density Array (TLDA) method to find the differentially expressed miRNAs, in the light of HIV-1 infected patients with low (<200 cell/ μ L), medium (200–350 cell/ μ L), and high (>350 cell/ μ L) CD4⁺ T cell count.

Results: Seven miRNAs (miR-29a, miR-223, miR-27a, miR-19b, miR-151-3p, miR-28-5p, miR-766 and miR-30a-3p) were significantly associated with CD4⁺ T cell count ($P < 0.05$) and thus have a great potential to serve as biomarkers for monitoring the HIV immune status. A combination of five miRNAs (miR-29a, miR-223, miR-27a, miR-19b, miR-151-3p) were found to distinguish the HIV-1 infected patients from healthy controls with sensitivity of 96.1% and specificity of 97.3% by RT-qPCR and receiver operational characteristic (ROC) curve analysis (AUC = 0.99).

Conclusions: We have identified highly sensitive and specific signatures of circulating miRNAs enabling non invasive detection and immune status monitoring of HIV-1 infection.

Keywords: HIV; miRNA; infection diagnosis; immune status

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Introduction

Over the past decade, acquired immunodeficiency syndrome (AIDS) caused by HIV-1 infection began to spread from the original drug use, commercial sex workers and other high-risk groups to the general population. By the end of 2014, nearly 501,000 HIV/AIDS people (including 59.1% HIV people and 40.9% AIDS patients) and 159,000 deaths had

been reported in China (1). However, HIV-1 is continuing to spread, with an estimated rate of about 50,000 new infections annually (1). Rapid and accurate diagnostic tools were essential for early detection and monitoring the immune status of HIV-1 infection. Current laboratory tests can detect HIV-1 antibodies, viral nucleic acids, CD4⁺ T lymphocytes, and p24 antigens (2). HIV-1 antibody test is the gold standard, but sometimes the false negative results

could be detected during the window period of infection. As the main target cells of HIV, the CD4⁺ T cell counts are useful to monitor how effective antiretroviral treatment (ART) is in suppressing the virus and determine the risk of progression of HIV disease.

Recently, several miRNAs are being used as biomarkers for the diagnosis of infectious diseases. MicroRNAs (miRNAs) are small (21–22 nt), non-coding RNAs which could play important roles in infectious diseases (3,4). Previous studies have found the different expression pattern of miRNAs in PBMCs, CD8⁺ T cells, monocytes and CD4⁺ T cells from HIV-1 infected subjects (5-9). Huang *et al.* has established that five miRNAs including miR-28 and miR-223 can directly target to the 3' end of HIV mRNAs in order to inhibit viral mRNA expression in CD4⁺ T cells (5). Wang *et al.* has reported that miR-223 and miR-28 showed different expression level between monocytes and macrophages in HIV infection (6). Other studies have shown that miR-29a in the PBMC and CD4⁺ T cells can reduce viral replication (10). Furthermore, circulating miRNAs have been intensively studied in various cancers and infectious diseases (11-16). Serum/plasma miRNAs are stable, resistant to RNase digestion and consistent in the same species which could act as useful biomarkers for disease detection (17,18).

The goal of our study was set to identify differentially expressed miRNAs isolated from HIV-1 individuals that could be used to assist in HIV-1 detection and analyze the potential biological functions of these miRNAs. To further illustrate the correlation between miRNAs expression and HIV/AIDS immune progression, we have separated the HIV-1 infected individuals into three groups according to the CD4⁺ T cell level and have analyzed the expression level of the candidate miRNAs in these three groups.

Methods

Sample collection

A total of 165 participants (37 healthy subjects and 128 patients with HIV-1 infection) were enrolled in the Jiangsu Province in 2013. The 128 infected people were categorized into low (CD4 <200 cell/ μ L) (n=41), medium (200 < CD4 <350 cell/ μ L) (n=44) and high (CD4 >350 cell/ μ L) (n=43) groups based on CD4⁺ T cell count. Among them, ten subjects (sex and age matched) from each group were used in the Taqman Low-Density Array (TLDA) study. All subjects taken by the RT-qPCR test were used

for confirming the array data. Blood samples were obtained by venipuncture into BD Vacutainer tubes with EDTA-k2. The samples were centrifuged immediately at 2,000 \times g for 15 minutes at room temperature (RT). Aliquots of plasma were stored in -80 °C until use. Plasma samples were collected from confirmed HIV-1 infected patients without ART who had HIV-1 antibody positive results using HIV Western Blot Assay (MP Biomedicals Asia Pacific Pte Ltd, Singapore) at the time of enrollment. All people were not infected with hepatitis B and C viruses

Analysis of the plasma miRNA profile by TLDA

The plasma pools were created by combining ten samples (30 μ L per sample) from four respective groups (control group; three HIV-1 infection groups with low, medium and high CD4⁺ T cells) for TLDA analysis. Total RNA was isolated from each pool of plasma samples using NucleoSpin miRNA Plasma kits (Macherey-Nagel GmbH & Co, Germany) following the manufacturer's instructions. miRNA expression profiles were executed by the TLDA v3.0 (Applied Biosystems, USA) which can detect 754 miRNAs including 4 endogenous controls. The TLDA experiment was performed and analyzed as previously reported (19).

Confirmation Candidate miRNAs using RT-qPCR

Total RNA used for RT-qPCR assay was extracted from individual plasma sample following the instruction as above. Cel-miR-238 used as an internal control was added into each individual sample before starting the isolation procedure. To confirm the array results, candidate miRNAs were quantified for each individual plasma sample by RT-qPCR test. The RT-qPCR experiment was performed and analyzed as previously reported (19).

Statistical analysis

Log₂ relative level was used to compare the difference between the target miRNA and cel-miR-238 (Δ Cq). Student's *t*-test was used to compare the differences in the miRNA expression between the two groups. 1-way ANOVA was used to compare between more than two groups and the differences between the groups were performed by the Fisher LSD test. A *p* value <0.05 shows the statistically significant. In addition, the area under curve (AUC) value and a receiver operating characteristic (ROC) curve were

Table 1 Demographic characteristics of HIV-1 infected patients and healthy controls

Sample characteristic	Patients group						Healthy controls group	
	CD4 ⁺ T cell counts (<200)		CD4 ⁺ T cell counts (200–350)		CD4 ⁺ T cell counts (>350)		TLDA study	Validation study
	TLDA study	Validation study	TLDA study	Validation study	TLDA study	Validation study		
Number of participants	10	41	10	44	10	43	10	37
Gender (male/female)	5/5	36/5	5/5	32/12	5/5	19/24	5/5	18/19
Age (years, mean ± SD)	33.50±8.71	42.54±16.93	34.30±8.76	41.52±12.72	34.90±8.86	44.05±13.42	34.50±10.11	39.92±11.59
CD4 ⁺ T cell count (cell/μL) (mean ± SD)	99.41±64.88		275.09±40.77		513.05±154.49		NA	

used to evaluate the diagnostic potential of each miRNA. 95% confidence intervals (CI) were used to detect the sensitivity and specificity of HIV-1 infection. In order to increase the accuracy of diagnostics, multiple logistic regression analysis was performed as previously reported (20).

Results and Discussion

Demographic characteristics of HIV-1 infected patient

Table 1 showed the demographic characteristics of HIV-1 infected patients. A total of 165 subjects were taken part in this study including 128 HIV-1 infected patients (87 males and 41 females; median age, 42.70±14.34 years) and 37 healthy people (18 males and 19 females; median age, 39.92±11.59 years). There was a significant difference ($P<0.05$, chi-square test) on the gender distribution between the HIV-1 infection and healthy people, but there was no significant difference in age between the two groups ($P>0.05$, *t*-test). The HIV-1 infected people were categorized into low (CD4 <200 cell/μL), medium (200 < CD4 <350 cell/μL) and high (CD4 >350 cell/μL) groups based on CD4⁺ T cell count. The first two groups showed significant differences ($P<0.01$ and $P<0.05$, chi-square test) in gender distribution between the HIV-1 infection and healthy people. There was no significant difference ($P>0.05$, chi-square test) in gender distribution in high CD4⁺ T cell count group. Although we found gender differences between the HIV-1 infection and healthy people, we and other study have shown that gender differences have no effect on miRNAs expression (15-17).

miRNA profiling analysis in control and HIV-1 Infected groups

TLDA analysis was executed to identify the differentially

expressed level in HIV-1 subjects with low, medium, high CD4⁺ T cell count (LTC, MTC, and HTC), and control subjects. Results from LTC, MTC, and HTC groups were compared with the control group. Of the 754 host miRNAs (including endogenous controls) incorporated in the array, 231, 345, 315, and 257 miRNAs (Cq values <40) were detected in plasma of healthy controls, LTC, MTC, and HTC groups, respectively. In order to screen out HIV-specific candidate miRNAs, we set up two criteria: (I) Cq values <35 in both of two groups; and (II) Fold change ≥ 2 between the two groups. Total of 150 miRNAs met the two criteria in LTC subjects, of which 147 were up-regulated and 3 were down-regulated compared to controls (Table S1). Similarly, in the MTC group, a total of 150 miRNAs (148 up-regulated and 2 down-regulated) were differentially regulated compared to healthy controls (Table S2). However, in the HTC group, only 117 miRNAs (115 up-regulated and 2 down-regulated) were differentially regulated compared to healthy controls (Table S3). Figure 1 displays the number and overlap of significantly dysregulated miRNAs which are specific to each group. A total of 112 miRNAs (LTC and HTC combined) showed different expression in HIV-1 infection compared with healthy controls (CT). Similarly, 145 miRNAs (LTC and MTC combined) and 113 miRNAs (MTC and HTC combined) were significantly dysregulated in the infection. Among the 150 miRNAs that were dysregulated between the controls and LTC groups, 4 were unique to the LTC group. When the control was compared with MTC and HTC separately, 3 of the 150 and 3 of the 117 miRNAs were specific to the MTC and HTC groups. While comparing all the three groups, there were 111 different expressed miRNAs in all of them. Based on the HIV infection literatures and the results of target gene analysis, 12 miRNAs (miR-29a, miR-

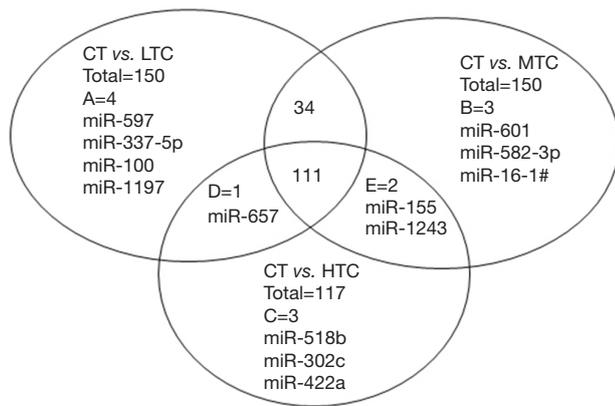


Figure 1 Expression profile of differentially regulated miRNAs in HIV-1 infected and controls. The Venn diagram displays the number and overlap of significantly differentially expressed miRNAs among the LTC, MTC, and HTC groups relative to the controls (CT) and within the infected groups.

223, miR-27a, miR-19b, miR-766, miR-28-5p, miR-151-3p, miR-30a-3p, miR125b, miR-18a, miR-1197 and miR-518b) were selected for further analysis. Among these, 9 miRNAs (miR-223, miR-19b, miR-27a, miR-30a-3p, miR-151-3p, miR-766, miR-28-5p, miR-125b, and miR-18a) were commonly up-regulated in all three groups. Mir-29a was up-regulated expression in LTC and MTC groups but not in HTC group. MiR-1197 and miR-518b were unique in LTC and HTC groups, separately.

RT-qPCR confirmation of miRNA expression in HIV-1 infected subjects

RT-qPCR (TaqMan miRNA assays) was used to confirm the expression levels of 12 candidate miRNAs which were identified by TLDA. Eight miRNAs (miR-29a, miR-19b, miR-223, miR-27a, miR-151-3p, miR-766, miR-28-5p, and miR-30a-3p) were significant up-regulation in HIV-1 infected plasma ($P < 0.05$, student's *t*-test) (Figure 2) compared to healthy control. However, there was no significant difference in other four miRNAs (miR-1197, miR-125b, miR18a, and miR-518b) in HIV-1 subjects compared to healthy controls (data not shown).

Diagnostic potential of plasma miRNAs

ROC curve analysis was performed to evaluate the diagnostic potential of candidate miRNAs. The ROC curves of miR-29a, miR-223, miR-27a, miR-19b and miR-151-3p

showed a high discrimination with AUC value of 0.949 (95% CI: 0.915-0.983), 0.905 (95% CI: 0.860-0.949), 0.897 (95% CI: 0.848-0.946), 0.989(95% CI: 0.974-1.003), 0.967(95% CI: 0.941-0.992), respectively (Figure 3). MiR-28-5p (95% CI: 0.703-0.859), miR-766 (95% CI: 0.694-0.842), and miR-30a-3p (95% CI: 0.680-0.834) showed a moderate discrimination with AUC value less than 0.8 (Figure 3). In order to increase the diagnostic efficiency of these markers, a combination of five miRNAs were used to show strong discrimination between the HIV-1 and control samples with high AUC value of 0.990 (Figure 4). Table 2 exhibits the specificity and sensitivity of each candidate miRNA with an optimal cutoff value. A cutoff value set at -6.00 , the combined miRNAs showed a specificity of 97.3% and a sensitivity of 96.1% (Table 2).

Different expression levels of eight miRNAs in LTC, MTC, and HTC groups

To monitor HIV/AIDS immune status, we further distinguished the expression levels of eight candidate miRNAs in LTC, MTC and HTC groups. The expression levels of miR-29a, miR-223, miR-27a, miR-19b, miR-151-3p, miR-766 and miR-30a-3p showed a significant difference among the LTC, MTC and HTC groups ($P < 0.05$, ANOVA test). However, there was no significant difference in the expression levels of miR-28a-5p among these three groups ($P > 0.05$, ANOVA test). Furthermore, multiple comparisons were carried out using the least significant difference (LSD) method. Figure 5 shows the different expression level of each miRNA in these three groups. Results from the comparison of the LTC and HTC groups indicated that six out of seven miRNAs (miR-29a, miR-151-3p, miR-223, miR-30a-3p, miR-19b, and miR-766) were significantly up-regulated ($P < 0.05$, LSD test) in HTC group while miR-27a had no significant difference. The comparison between LTC and MTC groups showed that all the seven miRNAs were significantly up-regulated in MTC group ($P < 0.05$, LSD test). While comparing MTC and HTC groups, only miR-27a showed a significant down-regulation in HTC group ($P < 0.001$, LSD test). Altogether, our results showed that the expression levels of seven candidate miRNAs are significantly associated with CD4⁺ T cell count. Previous study showed that several host miRNAs play an important role in disease development (21-23)). For example, Patel *et al.* indicated that the miR-29a expression level was higher in PBMC and plasma from asymptomatic person (high CD4⁺ T cells) in whom virus replication is

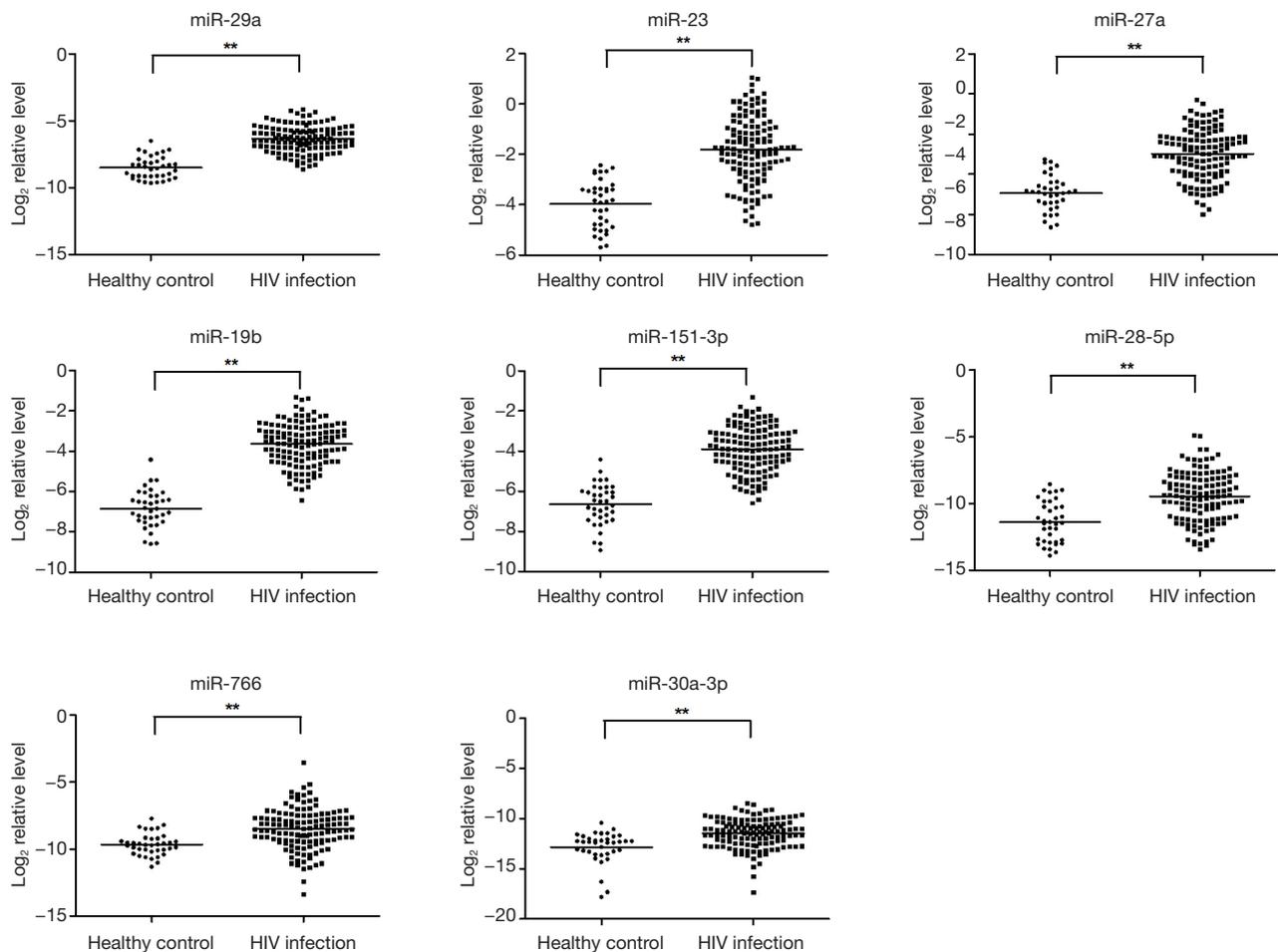


Figure 2 Eight miRNAs expression levels were analyzed in the plasma of HIV-1 infected subjects and healthy controls using RT-qPCR. Plasma levels of miR-29a, miR-223, miR-27a, miR-19b, miR-151-3p, miR-28-5p, miR-766, and miR-30a-3p were significantly higher in HIV infected subjects compared with those in the control group (**, $P < 0.01$). Expression levels of the miRNAs were normalized to cel-miR-238 (Log_2 relative level).

restricted, compared to symptomatic patients (low CD4^+ T cells) in whom there is active viral replication (24)). Our results have shown that miR-29a, miR-223, miR-19b, miR-151-3p, miR-766 and miR-30a-3p have higher expression pattern in plasma from HIV-1 infected person with high CD4^+ T cells compared to patients with low CD4^+ T cells. Thus we hypothesize that the expression level of these six miRNAs may increase during HIV-1 latency and decrease during active viral replication although the mechanisms are still unknown. We noted that the baseline of CD4^+ T cell count is very important. Compared with baseline $\text{CD4} < 200 \text{ cell}/\mu\text{L}$, HIV-1 infected person with baseline $\text{CD4} > 350 \text{ cell}/\mu\text{L}$ or $200 < \text{CD4} \leq 350 \text{ cell}/\mu\text{L}$ showed similar expression pattern of above six miRNAs. A previous study

has shown that patients with low level of CD4^+ T cell count ($\text{CD4} < 200 \text{ cell}/\mu\text{L}$) could impact immunological restoration (25). The CD4^+ T cells are very important in forming immune response during HIV infection. So, our study showed that these six miRNAs could serve as useful biomarkers for monitoring the immune status of HIV/AIDS progression.

More and more researchers have attracted the attention of the role of miRNAs in pathogen-host interactions. Human miRNAs involved in many biological process, molecular function, and regulation pathways. During HIV-1 infections, miRNAs can either affect viral replication by targeting HIV-1 directly or modulate the expression of host genes and pathways essential for it by targeting

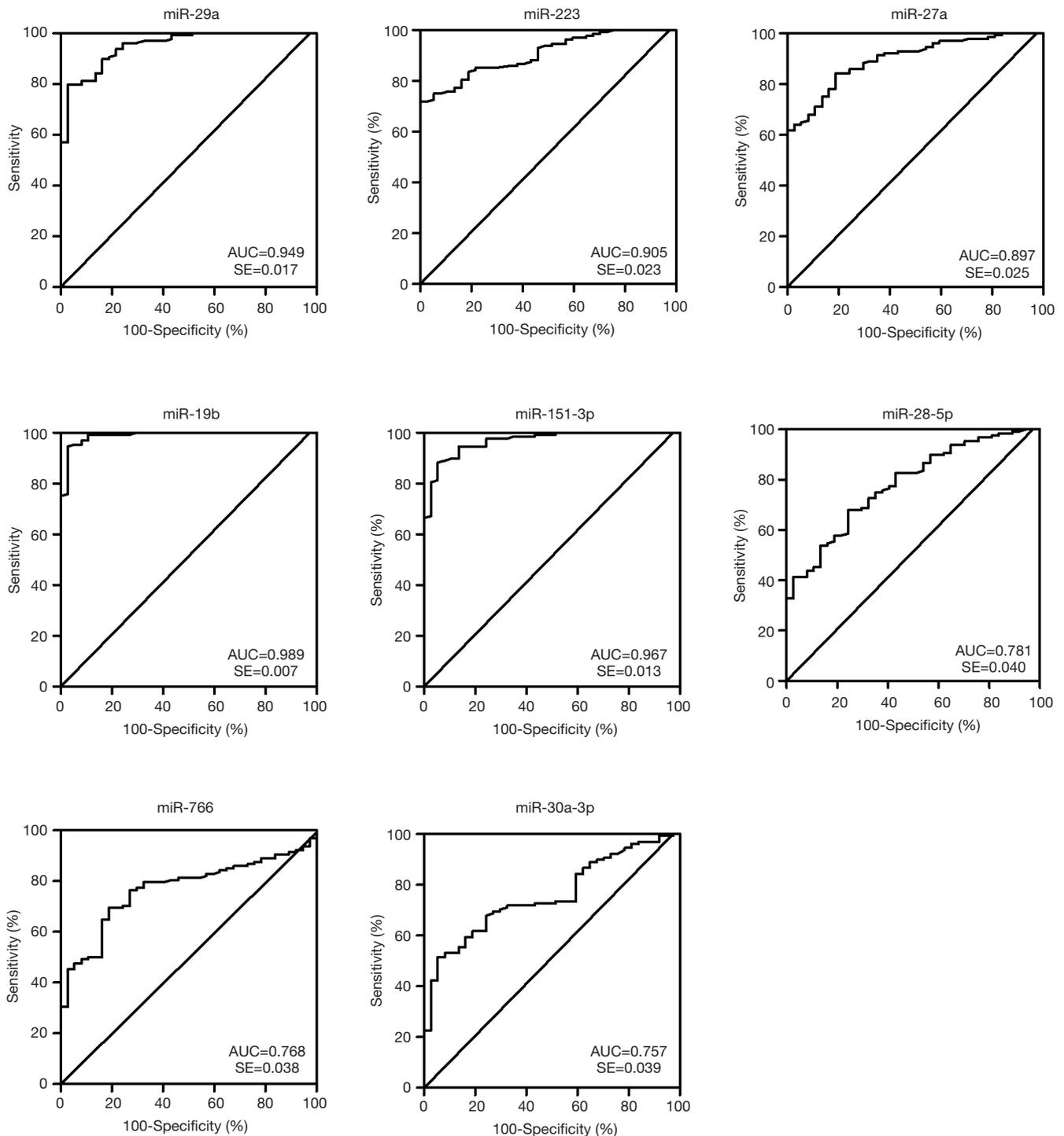


Figure 3 Receive operating characteristic (ROC) curves of differentially expressed miRNAs between HIV-1 infected subjects and healthy controls. ROC curves of miR-29a, miR-223, miR-27a, miR-19b, miR-151-3p, miR-28-5p, miR-766, and miR-30a-3p showed a different distinguishing efficiency.

host protein (26). For example, cellular miR-29a has been shown to inhibit HIV-1 replication by targeting the HIV-1 Nef transcripts (10,27). Also, cellular miR-28, miR-223, miR125b, miR-382 and miR-150 have been shown to be increased in resting primary CD4⁺ T cells compared to activated CD4⁺ T cells and can reduce HIV-1 replication by targeting HIV-1 mRNA transcripts (5). Our results also have shown a similar pattern of up-regulated miRNA expression such as miR-29a, miR-28 and miR-223 in plasma isolated from HIV-1 infected subjects. One study reported that miR-27a could decrease the phosphorylation of Akt and ERK, which could inhibit EV71 replication by targeting EGFR mRNA (28). The other study has demonstrated that miR-27a showed down-regulated in macrophages by targeting IL-10 through TLR2/4-driven inflammatory responses (29).

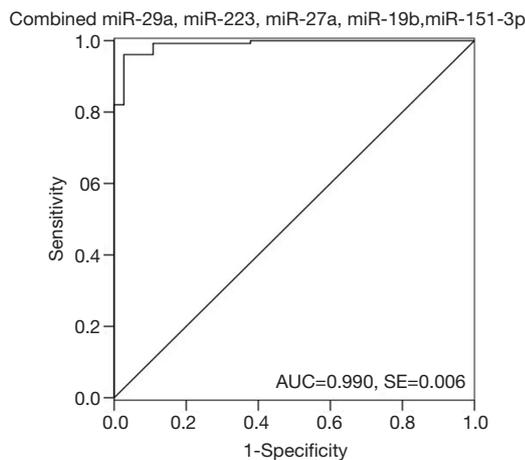


Figure 4 Receive operating characteristic (ROC) curves of differentially expressed miRNAs between HIV-1 infected subjects and healthy controls. The combination of the five miRNAs showed a higher AUC value of 0.990.

Only 5% people known as HIV controllers can maintain high levels of T cells without antiretroviral therapy for more than 5 years (30). HIV/AIDS may cause serious complications including serious pneumonia, central nervous system complications, opportunistic infections and various viral-induced cancers (31,32). Previous studies have shown that over-expression of miR-19b and miR-151 was strongly associated with cancer invasion and metastasis (33-35). However, the mechanisms remain largely unknown. For the first time, our study has shown that higher level of miR-19b and miR-151-3p were expressed in HIV-1 patient plasma compared with healthy controls. Further studies are needed to reveal the function of them in HIV-1 infected subjects.

Conclusions

TLDA assays identified the differential expression of 231, 345, 315, and 257 miRNAs in plasma of healthy controls and HIV-1 LTC, MTC, and HTC groups, respectively. 5 miRNAs (miR-29a, miR-223, miR-27a, miR-151-3p and miR-19b) was combined as a useful biomarker for simple and efficient detection of HIV-1 infection. The biological mechanisms of these miRNAs need further investigation. Our results showed that the expression level of seven candidate miRNAs (miR-29a, miR-223, miR-766, miR-19b, miR-151-3p, miR-27a, and miR-30a-3p) were significantly associated with CD4⁺ T cell count and thus may serve as biomarkers for monitoring the HIV/AIDS immune progression. However, there are several limitations in our study. Firstly, not all of dysregulated miRNAs were confirmed in present study and other miRNAs might serve as more efficient biomarkers. Secondly, a larger numbers of subjects are required to confirm our study.

Table 2 The sensitivity and specificity of candidate miRNAs and the combination of five miRNAs to diagnose HIV-1 infection with an optimal cutoff value

miRNA	Cutoff value	Sensitivity (%)	Specificity (%)
miR-29a	-7.13	79.7	97.3
miR-223	-2.42	71.9	100
miR-27a	-6.37	84.4	81.1
miR-19b	-5.43	94.5	97.3
miR-151-3p	-5.41	88.3	94.6
Combined miRNAs	-6.00	96.1	97.3

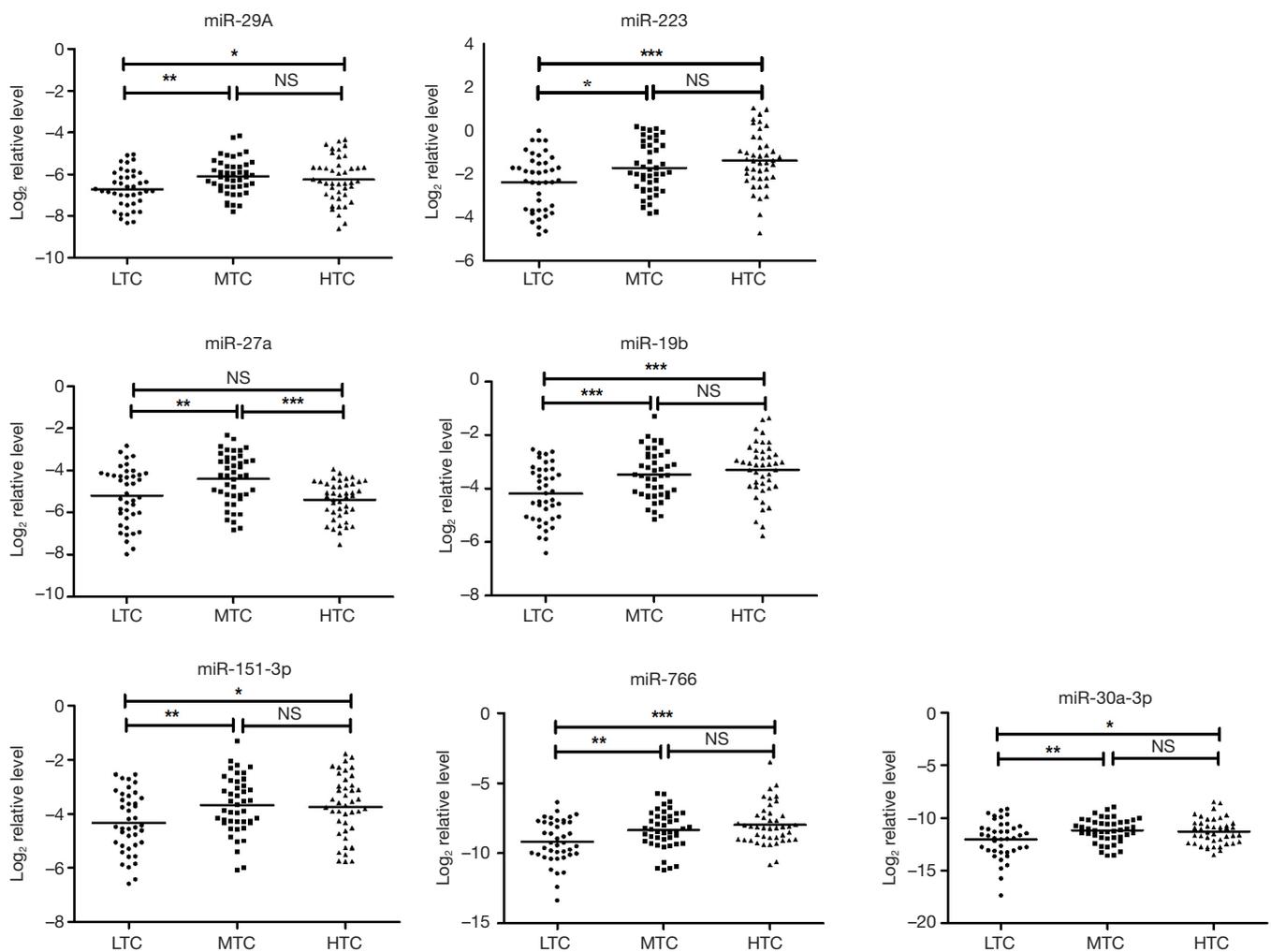


Figure 5 Different expression level of each miRNA in LTC (N=41), MTC (N=44) and HTC (N=43) groups using RT-qPCR. The least significant difference (LSD) method was used for pairwise comparison between all pairs of groups (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant). Expression levels of the miRNAs were normalized to cel-miR-238 (Log_2 relative level).

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Footnote

Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at <http://dx.doi.org/10.21037/jphe.2017.05.11>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study has been approved by the Ethics Committee of Jiangsu Provincial Center for Diseases Prevention and Control (approval number: JSCDCLL2013014). The written informed consent was

obtained from all participants.

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Supplementary

Table S1 Differential expressed miRNAs in HIV- infected subjects with CD4 <200 T⁺ cell count (LTC) compared with controls

miRNA name	Raw Cq for control	Raw Cq for LTC	Fold change
hsa-miR-597-4380960	32.44	19.81	34,848.40
<i>hsa-miR-151-3p-002254</i>	29.28	20.89	3,815.84
hsa-miR-409-3p-002332	29.71	23.99	595.88
hsa-miR-340#-002259	33.17	27.64	523.06
hsa-miR-224-4395210	33.52	27.55	344.02
hsa-miR-221-4373077	28.7	22.75	341.53
hsa-miR-130b-4373144	34.81	28.88	334.19
<i>hsa-miR-766-001986</i>	29.88	25	332.61
hsa-miR-331-3p-4373046	30.03	24.15	325.20
hsa-miR-425#-002302	33.36	28.58	310.21
hsa-miR-339-5p-4395368	34.68	28.9	301.75
hsa-miR-365-4373194	34.15	28.61	255.87
hsa-miR-22#-002301	33.72	29.24	253.05
hsa-miR-151-5P-002642	30.52	26.09	243.88
hsa-miR-652-4395463	32.22	26.82	232.24
hsa-miR-485-3p-4378095	32.32	27.01	218.12
hsa-miR-125a-5p-4395309	34.83	29.53	215.81
<i>hsa-miR-30a-3p-000416</i>	32.34	28.14	207.22
hsa-miR-505#-002087	34.69	30.61	190.61
hsa-miR-126#-000451	28.51	24.52	179.26
hsa-miR-132-4373143	32.92	27.94	173.42
hsa-miR-30e-3p-000422	29.21	25.31	168.84
hsa-miR-15b-4373122	28.36	23.43	167.08
hsa-miR-532-5p-4380928	32.1	27.2	164.14
hsa-miR-199a-3p-4395415	28.5	23.6	163.67
hsa-miR-625#-002432	30.39	26.58	158.85
hsa-miR-425-4380926	34.69	29.9	151.73
hsa-let-7d-4395394	28.97	24.2	150.12
hsa-miR-324-5p-4373052	31.56	26.8	149.04
hsa-miR-127-3p-4373147	30.89	26.22	139.78
hsa-miR-584-001624	34.22	30.61	138.50
hsa-miR-122-4395356	27.98	23.36	135.62
hsa-miR-195-4373105	29.69	25.13	130.28
hsa-miR-339-3p-4395295	33.87	29.38	123.93
hsa-miR-27b-4373068	30.4	25.92	122.43
hsa-miR-15a#-002419	34.61	31.19	121.47
<i>hsa-miR-28-5p-4373067</i>	30.36	25.97	114.79
hsa-miR-744-4395435	31.37	27.08	108.06
hsa-miR-93#-002139	31.3	28.11	103.48
hsa-miR-18a-4395533	29.5	25.29	102.32
hsa-miR-24-4373072	24.87	20.66	102.03
hsa-miR-382-4373019	33.77	29.57	101.70
hsa-miR-491-5p-4381053	34.43	30.23	100.61
hsa-miR-26a-4395166	27.34	23.15	100.33
hsa-miR-192-4373108	32.4	28.24	98.52
hsa-miR-25-4373071	27.4	23.27	96.02
hsa-miR-495-4381078	31.25	27.14	95.05
hsa-let-7g-4395393	30.74	26.63	94.89
<i>hsa-miR-27a-4373287</i>	28.51	24.41	94.20
hsa-miR-598-4395179	33.9	29.84	91.38
hsa-miR-148b-4373129	31.36	27.32	90.37
hsa-miR-223#-002098	31.42	28.52	84.32
hsa-let-7a-4373169	30.62	26.71	83.11
hsa-miR-671-3p-4395433	33.95	30.04	82.84
hsa-miR-328-4373049	28.62	24.71	82.75
hsa-miR-146a-4373132	25.85	21.96	81.67
hsa-miR-574-3p-4395460	29.06	25.18	81.34
hsa-miR-376c-4395233	32.17	28.31	80.02
hsa-miR-222-4395387	27.45	23.62	78.40
hsa-miR-191-4395410	25.37	21.54	78.28
hsa-miR-376a-4373026	32.04	28.3	73.34
hsa-miR-375-4373027	30.76	27.04	72.64
hsa-miR-324-3p-4395272	32.99	29.3	70.89
hsa-let-7b-4395446	30.12	26.45	70.13
hsa-miR-301a-4373064	30.56	26.91	69.08
hsa-miR-20a-4373286	26.01	22.39	67.56
<i>hsa-miR-125b-4373148</i>	33.23	29.67	64.54
hsa-miR-145-4395389	28.95	25.4	64.43
hsa-miR-140-5p-4373374	29.86	26.35	62.83
hsa-miR-885-5p-4395407	30.48	26.97	62.74
hsa-miR-148a-4373130	29.46	25.97	61.77
hsa-miR-142-3p-4373136	26.95	23.46	61.75
hsa-miR-335-4373045	28.85	25.39	60.57
hsa-miR-345-4395297	31.22	27.79	59.48
hsa-miR-30d-000420	28.73	26.34	59.11
hsa-let-7e-4395517	28	24.65	55.98
hsa-miR-30a-5p-000417	25.2	22.93	54.81
hsa-miR-93-4373302	27.1	23.8	54.15
hsa-miR-17-4395419	23.97	20.68	53.77
hsa-miR-16-4373121	25.2	21.96	52.03
hsa-miR-152-4395170	29.09	25.86	51.62
hsa-miR-340-4395369	30.89	27.71	49.99
hsa-miR-26b-4395167	29.11	25.93	49.94
hsa-miR-99b-4373007	32.29	29.13	49.02
<i>hsa-miR-223-4395406</i>	19.61	16.46	48.72
hsa-miR-21-4373090	25.68	22.54	48.58
hsa-miR-30b-4373290	26	22.87	48.03
hsa-miR-197-4373102	31.67	28.56	47.48
hsa-miR-139-5p-4395400	28.9	25.8	47.32
hsa-miR-106a-4395280	24.08	20.99	47.13
hsa-miR-185-4395382	31.18	28.11	46.30
hsa-miR-374b-4381045	28.06	25	46.07
hsa-miR-374a-4373028	30.06	27	45.75
<i>hsa-miR-19b-4373098</i>	24.42	21.44	43.31
hsa-miR-320B-002844	30.31	28.45	41.12
hsa-miR-146b-5p-4373178	28.19	25.34	39.83
hsa-miR-28-3p-4395557	28.87	26.11	37.16
hsa-miR-590-5p-4395176	31.14	28.44	35.94
hsa-miR-484-4381032	22.87	20.21	34.64
hsa-miR-136-4373173	34.7	32.09	33.63
hsa-miR-338-5P-002658	34.29	32.75	33.05
hsa-miR-126-4395339	23.51	20.98	31.84
hsa-miR-128-4395327	29.63	27.09	31.84
hsa-miR-10a-4373153	32.97	30.51	30.37
hsa-miR-20b-4373263	27.94	25.49	30.03
hsa-miR-218-4373081	31.36	28.93	29.70
hsa-miR-720-002895	26.34	24.94	29.67
hsa-miR-106b-4373155	26.19	23.77	29.47
hsa-miR-186-4395396	28.87	26.49	28.49
hsa-miR-361-5p-4373035	32.86	30.49	28.42
hsa-miR-30c-4373060	24.52	22.19	27.78
hsa-miR-92a-4395169	23.58	21.25	27.74
hsa-miR-10b#-002315	34.54	33.26	27.44
hsa-miR-454-4395434	31.3	28.99	27.42
hsa-miR-101-4395364	31.44	29.14	27.19
hsa-miR-194-4373106	33.45	31.17	26.74
<i>hsa-miR-29a-4395223</i>	29.06	26.8	26.43
hsa-miR-642-4380995	34.17	31.91	26.38
hsa-miR-660-4380925	30.17	27.91	26.35
hsa-miR-9#-002231	32.02	30.84	25.54
hsa-miR-451-4373360	24.3	22.19	23.65
hsa-miR-200c-4395411	34.73	32.67	23.03
hsa-miR-378-002243	30.22	29.2	22.99
hsa-miR-423-5p-4395451	31.26	29.21	22.74
hsa-miR-193b-4395478	32.64	30.63	22.13
hsa-miR-204-4373094	32.41	30.49	20.75
hsa-miR-744#-002325	32.75	31.97	19.33
hsa-miR-133a-4395357	30.1	28.34	18.71
hsa-miR-579-4395509	32.89	31.18	18.02
hsa-miR-1274B-002884	22.62	22.02	17.14
hsa-miR-1290-002863	31.62	31.06	16.73
hsa-miR-320-4395388	25.31	23.74	16.40
hsa-miR-19a-4373099	28.74	27.25	15.48
hsa-miR-362-3p-4395228	33.86	32.41	14.98
hsa-miR-144#-002148	30	29.73	13.67
hsa-miR-193a-5p-4395392	29.99	28.73	13.20
hsa-miR-181a-4373117	33.57	32.49	11.64
hsa-miR-337-5p-4395267	34.72	33.68	11.32
hsa-miR-483-5p-4395449	30.29	29.29	10.94
hsa-miR-342-3p-4395371	27.61	26.67	10.50
hsa-miR-1255B-002801	32.51	33.56	5.48
hsa-miR-367-4373034	31.01	31.05	5.37
hsa-miR-150-4373127	24.56	24.72	4.94
hsa-miR-486-5p-4378096	29.65	29.82	4.89
hsa-miR-657-001512	33.43	34.73	4.60
hsa-miR-381-4373020	20.47	20.92	4.04
hsa-miR-100-4373160	30.51	31.53	2.71
hsa-miR-661-001606	28.01	34.7	0.11
<i>hsa-miR-1197-002810</i>	23.45	33.87	0.01
hsa-miR-1274A-002883	12.73	27.43	0.00

The Fold change between two groups was calculated by $2^{-\Delta\Delta Cq}$ method: $\Delta\Delta Cq = \Delta Cq_{\text{HIV infection}} - \Delta Cq_{\text{control}}$; $\Delta Cq = Cq_{\text{target miRNA}} - Cq_{\text{US}}$. Italic labeled miRNAs were used for RT-qPCR confirmation test.

Table S2 Differential expressed miRNAs in HIV-infected subjects with 200< CD4 <350 T⁺ cell count (MTC) compared with controls

miRNA name	Raw Cq for control	Raw Cq for MTC	Fold change
<i>hsa-miR-151-3p-002254</i>	29.28	24.17	413.37
hsa-miR-125a-5p-4395309	34.83	28.16	295.93
hsa-miR-340#-002259	33.17	29.23	183.29
hsa-miR-409-3p-002332	29.71	25.94	163.87
hsa-miR-130b-4373144	34.81	29.31	131.40
hsa-miR-151-5P-002642	30.52	27.16	122.80
hsa-miR-10b#-002315	34.54	31.24	117.62
hsa-miR-126#-000451	28.51	25.23	115.64
hsa-miR-22#-002301	33.72	30.52	110.04
<i>hsa-miR-766-001986</i>	29.88	26.69	109.48
<i>hsa-miR-30a-3p-000416</i>	32.34	29.22	103.56
hsa-miR-625#-002432	30.39	27.28	103.31
hsa-miR-584-001624	34.22	31.18	98.65
hsa-miR-365-4373194	34.15	29.1	96.27
hsa-miR-331-3p-4373046	30.03	25.01	94.76
hsa-miR-192-4373108	32.4	27.45	90.12
hsa-miR-224-4395210	33.52	28.63	86.32
hsa-miR-532-5p-4380928	32.1	27.24	84.96
hsa-miR-15a#-002419	34.61	31.95	75.84
hsa-miR-339-5p-4395368	34.68	29.99	75.17
hsa-miR-132-4373143	32.92	28.47	63.35
hsa-miR-425#-002302	33.36	31.02	60.52
hsa-miR-652-4395463	32.22	27.85	60.05
hsa-miR-30e-3p-000422	29.21	26.95	57.30
hsa-miR-30a-5p-000417	25.2	23.08	52.07
hsa-miR-221-4373077	28.7	24.55	51.60
hsa-miR-199a-3p-4395415	28.5	24.36	50.98
hsa-miR-25-4373071	27.4	23.28	50.37
hsa-miR-18a-4395533	29.5	25.44	48.72
hsa-miR-495-4381078	31.25	27.2	48.03
hsa-miR-30d-000420	28.73	26.74	47.40
hsa-miR-485-3p-4378095	32.32	28.33	46.21
hsa-miR-1243-002854	19.76	17.84	45.34
hsa-miR-15b-4373122	28.36	24.46	43.15
hsa-miR-324-3p-4395272	32.99	29.12	42.48
hsa-miR-375-4373027	30.76	26.91	42.15
hsa-miR-598-4395179	33.9	30.06	41.42
hsa-miR-324-5p-4373052	31.56	27.78	39.92
hsa-miR-195-4373105	29.69	25.92	39.80
hsa-miR-320B-002844	30.31	28.6	39.03
hsa-miR-24-4373072	24.87	21.13	38.74
hsa-miR-93#-002139	31.3	29.61	38.69
<i>hsa-miR-19b-4373098</i>	24.42	20.69	38.41
hsa-miR-223#-002098	31.42	29.79	37.04
hsa-miR-339-3p-4395295	33.87	30.25	35.94
<i>hsa-miR-27a-4373287</i>	28.51	24.91	35.41
hsa-miR-491-5p-4381053	34.43	30.85	34.80
hsa-miR-671-3p-4395433	33.95	30.39	34.35
<i>hsa-miR-28-5p-4373067</i>	30.36	26.8	34.29
hsa-miR-16-4373121	25.2	21.66	33.78
hsa-miR-505#-002087	34.69	33.2	33.67
hsa-miR-642-4380995	34.17	30.65	33.51
hsa-miR-26a-4395166	27.34	23.83	33.24
hsa-miR-20a-4373286	26.01	22.58	31.20
hsa-miR-338-5P-002658	34.29	32.93	30.80
hsa-miR-26b-4395167	29.11	25.75	29.92
<i>hsa-miR-125b-4373148</i>	33.23	29.89	29.31
hsa-let-7d-4395394	28.97	25.64	29.27
hsa-miR-144#-002148	30	28.72	29.20
hsa-miR-140-5p-4373374	29.86	26.54	29.02
hsa-miR-145-4395389	28.95	25.63	29.00
hsa-let-7g-4395393	30.74	27.43	28.90
hsa-miR-660-4380925	30.17	26.87	28.57
hsa-miR-142-3p-4373136	26.95	23.68	27.96
hsa-miR-136-4373173	34.7	31.45	27.73
hsa-miR-127-3p-4373147	30.89	27.72	26.17
hsa-miR-328-4373049	28.62	25.47	25.79
hsa-miR-374a-4373028	30.06	26.93	25.34
hsa-miR-93-4373302	27.1	23.97	25.32
hsa-miR-27b-4373068	30.4	27.3	24.83
hsa-miR-185-4395382	31.18	28.13	24.07
hsa-miR-345-4395297	31.22	28.19	23.75
hsa-miR-222-4395387	27.45	24.44	23.38
hsa-miR-146a-4373132	25.85	22.84	23.37
hsa-miR-122-4395356	27.98	24.98	23.32
hsa-miR-148a-4373130	29.46	26.47	23.13
hsa-miR-744-4395435	31.37	28.39	22.94
hsa-miR-376c-4395233	32.17	29.21	22.68
hsa-miR-21-4373090	25.68	22.72	22.63
hsa-miR-301a-4373064	30.56	27.64	21.93
hsa-miR-148b-4373129	31.36	28.46	21.69
hsa-miR-181a-4373117	33.57	30.68	21.59
hsa-miR-376a-4373026	32.04	29.2	20.83
hsa-miR-451-4373360	24.3	21.47	20.65
hsa-miR-191-4395410	25.37	22.57	20.18
hsa-miR-340-4395369	30.89	28.14	19.58
hsa-miR-30b-4373290	26	23.3	18.91
hsa-miR-378-002243	30.22	29.57	18.77
hsa-miR-590-5p-4395176	31.14	28.46	18.64
hsa-miR-99b-4373007	32.29	29.62	18.52
hsa-miR-19a-4373099	28.74	26.07	18.51
hsa-miR-744#-002325	32.75	32.15	18.08
<i>hsa-miR-223-4395406</i>	19.61	17.06	17.01
hsa-miR-92a-4395169	23.58	21.03	16.98
hsa-miR-106a-4395280	24.08	21.54	16.94
hsa-miR-17-4395419	23.97	21.44	16.73
hsa-miR-20b-4373263	27.94	25.44	16.42
hsa-miR-425-4380926	34.69	32.19	16.37
hsa-miR-361-5p-4373035	32.86	30.39	16.18
hsa-miR-146b-5p-4373178	28.19	25.73	15.98
hsa-miR-335-4373045	28.85	26.42	15.70
hsa-let-7e-4395517	28	25.57	15.62
hsa-miR-197-4373102	31.67	29.27	15.40
hsa-miR-885-5p-4395407	30.48	28.08	15.37
hsa-miR-454-4395434	31.3	28.92	15.18
hsa-miR-374b-4381045	28.06	25.69	15.07
hsa-miR-139-5p-4395400	28.9	26.57	14.65
hsa-miR-101-4395364	31.44	29.12	14.55
<i>hsa-miR-29a-4395223</i>	29.06	26.76	14.33
hsa-miR-582-3p-4395510	34.91	32.62	14.24
hsa-let-7b-4395446	30.12	27.84	14.16
hsa-miR-10a-4373153	32.97	30.76	13.46
hsa-miR-106b-4373155	26.19	24.01	13.13
hsa-miR-579-4395509	32.89	30.73	13.04
hsa-miR-126-4395339	23.51	21.38	12.77
hsa-miR-484-4381032	22.87	20.76	12.49
hsa-miR-128-4395327	29.63	27.53	12.40
hsa-miR-574-3p-4395460	29.06	26.98	12.37
hsa-miR-194-4373106	33.45	31.37	12.26
hsa-let-7a-4373169	30.62	28.59	11.89
hsa-miR-186-4395396	28.87	26.92	11.19
hsa-miR-152-4395170	29.09	27.16	11.09
hsa-miR-382-4373019	33.77	31.88	10.84
hsa-miR-193a-5p-4395392	29.99	28.1	10.76
hsa-miR-30c-4373060	24.52	22.65	10.63
hsa-miR-1274B-002884	22.62	22.85	10.19
hsa-miR-193b-4395478	32.64	30.91	9.62
hsa-miR-9#-002231	32.02	32.45	8.86
hsa-miR-28-3p-4395557	28.87	27.32	8.49
hsa-miR-1290-002863	31.62	32.28	7.59
hsa-miR-720-002895	26.34	27.02	7.45
hsa-miR-423-5p-4395451	31.26	29.93	7.28
hsa-miR-483-5p-4395449	30.29	28.98	7.17
hsa-miR-342-3p-4395371	27.61	26.34	6.98
hsa-miR-367-4373034	31.01	29.77	6.87
hsa-miR-16-1#-002420	33.63	34.52	6.49
hsa-miR-320-4395388	25.31	24.16	6.44
hsa-miR-150-4373127	24.56	23.46	6.23
hsa-miR-486-5p-4378096	29.65	28.64	5.84
hsa-miR-601-001558	32.97	34.04	5.69
hsa-miR-362-3p-4395228	33.86	32.9	5.66
hsa-miR-200c-4395411	34.73	33.89	5.21
hsa-miR-133a-4395357	30.1	29.39	4.76
hsa-miR-204-4373094	32.41	32.1	3.61
hsa-miR-1255B-002801	32.51	34.32	3.41
hsa-miR-661-001606	28.01	30.09	2.83
hsa-miR-381-4373020	20.47	20.77	2.37
hsa-miR-218-4373081	31.36	31.88	2.03
hsa-miR-155-4395459	24.15	26.9	0.43
hsa-miR-1274A-002883	12.73	28.92	0.00

The Fold change between two groups was calculated by $2^{-\Delta\Delta Cq}$ method: $\Delta\Delta Cq = \Delta Cq_{HIV\ infection} - \Delta Cq_{control}$; $\Delta Cq = Cq_{target\ miRNA} - Cq_{UE}$. Italic labeled miRNAs were used for RT-qPCR confirmation test.

Table S3 Differential expressed miRNAs in HIV- infected subjects with CD4 >350 T⁺ cell count (HTC) compared with controls

miRNA name	Raw Cq for control	Raw Cq for HTC	Fold change
<i>hsa-miR-151-3p-002254</i>	29.28	22.02	2,539.61
<i>hsa-miR-766-001986</i>	29.88	26.92	129.22
hsa-miR-1243-002854	19.76	16.85	124.63
hsa-miR-340#-002259	33.17	30.78	86.48
hsa-miR-505#-002087	34.69	32.55	73.16
hsa-miR-409-3p-002332	29.71	27.71	66.10
hsa-miR-625#-002432	30.39	28.72	52.72
hsa-miR-338-5P-002658	34.29	32.92	42.88
hsa-miR-425#-002302	33.36	32.31	34.29
hsa-miR-22#-002301	33.72	32.69	33.80
hsa-miR-126#-000451	28.51	27.51	33.11
hsa-miR-224-4395210	33.52	30.2	27.81
hsa-miR-151-5P-002642	30.52	29.82	26.94
hsa-miR-365-4373194	34.15	31.1	23.11
hsa-miR-125a-5p-4395309	34.83	31.81	22.58
hsa-miR-30e-3p-000422	29.21	28.87	20.93
hsa-miR-320B-002844	30.31	30.18	18.15
hsa-let-7b-4395446	30.12	27.5	17.15
hsa-miR-485-3p-4378095	32.32	29.7	17.09
hsa-miR-367-4373034	31.01	28.65	14.38
hsa-miR-328-4373049	28.62	26.27	14.22
<i>hsa-miR-30a-3p-000416</i>	32.34	32.58	14.01
<i>hsa-miR-518b-4373246</i>	31.67	29.4	13.51
hsa-miR-25-4373071	27.4	25.17	13.08
hsa-miR-324-5p-4373052	31.56	29.35	12.94
hsa-miR-221-4373077	28.7	26.51	12.75
hsa-miR-192-4373108	32.4	30.25	12.39
hsa-miR-324-3p-4395272	32.99	30.89	11.95
hsa-miR-671-3p-4395433	33.95	31.93	11.34
hsa-miR-30a-5p-000417	25.2	25.75	11.32
hsa-miR-652-4395463	32.22	30.2	11.29
hsa-miR-199a-3p-4395415	28.5	26.59	10.43
hsa-miR-331-3p-4373046	30.03	28.2	9.95
<i>hsa-miR-28-5p-4373067</i>	30.36	28.55	9.74
hsa-let-7d-4395394	28.97	27.18	9.69
hsa-miR-375-4373027	30.76	28.99	9.53
hsa-miR-145-4395389	28.95	27.19	9.42
hsa-miR-223#-002098	31.42	32.3	9.03
hsa-miR-744-4395435	31.37	29.7	8.87
hsa-miR-598-4395179	33.9	32.26	8.69
hsa-miR-93#-002139	31.3	32.27	8.47
hsa-miR-144#-002148	30	30.98	8.43
hsa-miR-127-3p-4373147	30.89	29.34	8.19
hsa-miR-136-4373173	34.7	33.19	7.97
hsa-miR-122-4395356	27.98	26.47	7.96
<i>hsa-miR-125b-4373148</i>	33.23	31.72	7.92
hsa-let-7e-4395517	28	26.5	7.87
hsa-let-7g-4395393	30.74	29.25	7.83
hsa-miR-18a-4395533	29.5	28.04	7.69
hsa-miR-197-4373102	31.67	30.24	7.53
hsa-miR-1274B-002884	22.62	23.77	7.46
hsa-miR-484-4381032	22.87	21.45	7.46
hsa-miR-195-4373105	29.69	28.33	7.15
hsa-miR-376a-4373026	32.04	30.69	7.11
hsa-miR-495-4381078	31.25	29.95	6.85
hsa-miR-491-5p-4381053	34.43	33.22	6.44
hsa-miR-92a-4395169	23.58	22.38	6.42
hsa-miR-133a-4395357	30.1	28.91	6.36
hsa-miR-142-3p-4373136	26.95	25.8	6.18
hsa-miR-26a-4395166	27.34	26.22	6.08
hsa-miR-15b-4373122	28.36	27.24	6.02
hsa-miR-657-001512	33.43	34.9	5.96
hsa-miR-885-5p-4395407	30.48	29.39	5.94
hsa-miR-454-4395434	31.3	30.22	5.91
hsa-miR-130b-4373144	34.81	33.77	5.73
hsa-miR-30d-000420	28.73	30.3	5.56
hsa-miR-10a-4373153	32.97	31.98	5.55
hsa-miR-93-4373302	27.1	26.14	5.41
hsa-miR-532-5p-4380928	32.1	31.21	5.19
hsa-miR-1290-002863	31.62	33.3	5.17
hsa-miR-486-5p-4378096	29.65	28.78	5.10
hsa-miR-574-3p-4395460	29.06	28.2	5.09
hsa-miR-24-4373072	24.87	24	5.09
hsa-miR-381-4373020	20.47	19.63	4.98
hsa-let-7a-4373169	30.62	29.79	4.97
hsa-miR-222-4395387	27.45	26.63	4.92
hsa-miR-361-5p-4373035	32.86	32.08	4.81
hsa-miR-720-002895	26.34	28.14	4.73
hsa-miR-339-5p-4395368	34.68	33.93	4.68
hsa-miR-193b-4395478	32.64	31.9	4.66
<i>hsa-miR-27a-4373287</i>	28.51	27.85	4.42
hsa-miR-660-4380925	30.17	29.51	4.40
hsa-miR-26b-4395167	29.11	28.49	4.31
hsa-miR-16-4373121	25.2	24.6	4.23
hsa-miR-30b-4373290	26	25.43	4.14
hsa-miR-642-4380995	34.17	33.65	4.00
hsa-miR-378-002243	30.22	32.28	3.98
hsa-miR-423-5p-4395451	31.26	30.85	3.70
hsa-miR-20a-4373286	26.01	25.61	3.67
hsa-miR-193a-5p-4395392	29.99	29.61	3.62
hsa-miR-302c-4378072	19.6	19.26	3.52
hsa-miR-27b-4373068	30.4	30.08	3.47
hsa-miR-345-4395297	31.22	30.93	3.41
hsa-miR-382-4373019	33.77	33.49	3.39
hsa-miR-146a-4373132	25.85	25.6	3.30
hsa-miR-99b-4373007	32.29	32.05	3.29
hsa-miR-28-3p-4395557	28.87	28.64	3.25
hsa-miR-181a-4373117	33.57	33.44	3.06
hsa-miR-374a-4373028	30.06	29.95	3.02
<i>hsa-miR-19b-4373098</i>	24.42	24.33	2.97
hsa-miR-191-4395410	25.37	25.32	2.88
hsa-miR-148a-4373130	29.46	29.44	2.84
<i>hsa-miR-223-4395406</i>	19.61	19.6	2.81
hsa-miR-128-4395327	29.63	29.63	2.78
hsa-miR-152-4395170	29.09	29.12	2.73
hsa-miR-422a-4395408	34.78	34.82	2.71
hsa-miR-451-4373360	24.3	24.36	2.66
hsa-miR-150-4373127	24.56	24.63	2.66
hsa-miR-21-4373090	25.68	25.78	2.60
hsa-miR-139-5p-4395400	28.9	29.17	2.32
hsa-miR-30c-4373060	24.52	24.81	2.29
hsa-miR-301a-4373064	30.56	30.87	2.25
hsa-miR-140-5p-4373374	29.86	30.2	2.21
hsa-miR-106a-4395280	24.08	24.49	2.10
hsa-miR-374b-4381045	28.06	28.5	2.05
has-miR-155-4395459	24.15	27.64	0.25
hsa-miR-1274A-002883	12.73	33.07	0.00

The Fold change between two groups was calculated by $2^{-\Delta\Delta Cq}$ method: $\Delta\Delta Cq = \Delta Cq_{HIV\ infection} - \Delta Cq_{control}$; $\Delta Cq = Cq_{target\ miRNA} - Cq_{UB}$. Italic labeled miRNAs were used for RT-qPCR confirmation test.