Transcriptomic Analysis of Peripheral Blood Mononuclear Cells in Rapid Progressors in Early HIV Infection Identifies a Signature Closely Correlated with Disease Progression

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BACKGROUND: A substantial percentage (10%–15%) of HIV-infected individuals experience a sharp decline in CD4+ T-cell counts and progress to AIDS quickly after primary infection. Identification of biomarkers distinguishing rapid progressors (RPs) vs chronic progressors (CPs) is critical for early clinical intervention and could provide novel strategies to facilitate vaccine design and immune therapy.

METHODS: mRNA and microRNA (miRNA) expression profiles in the peripheral blood mononuclear cells (PBMCs) of RPs and CPs were investigated at 111 (22) days [mean (SD)] of HIV infection. The association of mRNA and miRNA expression with disease progression was examined by ROC analysis and Kaplan–Meier survival analysis.

RESULTS: Pathway enrichment analysis showed that genes with deregulated expression in RPs were primarily involved in apoptosis pathways. Furthermore, we found that 5 miRNAs (miR-31, −200c, −526a, −99a, and −503) in RPs were significantly decreased compared to those in CPs (P < 0.05). The decreased expression of these miRNAs was associated with a rapid disease of progression of HIV infection with a 94% predictive value as measured by the area under the curve. The upregulated predicted targets from the 5 signature miRNAs and all upregulated genes identified from mRNA microarray analysis converged to the apoptosis pathway. Moreover, overexpression of miR-31 in primary human T cells promoted their survival.

CONCLUSIONS: Our results have identified a distinct transcriptomic signature in PBMCs of RPs and provided novel insights to the pathogenesis of HIV infection.

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Multiple longitudinal studies have revealed dramatic variation in the outcome of untreated HIV-1 infection, ranging from rapid AIDS development in under a year to no progression decades later (1). The unique pathogenic development of HIV-infected patients with rapid progression, representing 10%–15% of the HIV-positive population (2), remains less understood than those with chronic progression or elite controllers. Limited studies previously carried out with this patient population have identified several viral, genetic, and immunologic factors that are associated with rapid disease progression, such as the early emergence of CXCR4-utilizing HIV strains, risk single-nucleotide polymorphisms (SNPs)3 within interleukin-7Rα or Toll-like receptor loci, and high concentrations of IP-10 (interferon γ induced protein) in the plasma (3–7). Several months after HIV seroconversion, plasma viremia reaches a steady state or viral set point, which is an important determinant of the rate of HIV disease progression (8). However, to date, it is still unclear whether the immune system of rapid progressors (RPs) has been uniquely reprogrammed to gain intrinsic distinction from other patients upon HIV infection, especially during early HIV infection (EHI).

Transcriptomic studies in HIV infection have revealed global changes in gene expression across the majority of cell populations in the innate and acquired immune systems (9). Besides reshaping the expression...
of protein-coding genes, transcriptional reprogramming during HIV infection also alters microRNA (miRNA) expression profiles (10). Interferon-stimulated genes, genes controlling T-cell differentiation, cell cycle, and apoptosis, as well as several miRNA signatures, have been shown to contribute to the distinct clinical outcomes of HIV infection (11–16). However, the pattern of the transcriptomic profile associated with HIV disease progression remains largely elusive and the miRNA profiles of peripheral blood mononuclear cells (PBMCs) from RPs have yet to be examined (16–18).

Since 2008, we have been recruiting a large-scale open prospective cohort from HIV voluntary counseling and testing centers. The exact infection time for acute HIV patients was determined by using a third-generation ELISA antibody test and pooled nucleic acid amplification testing for HIV screening (19). In this cohort, we found that within 1 year of infection, a significant portion of acute HIV-infected patients exhibited a dramatic decline in their CD4+ T-cell counts, to 350 cells/µL, the recommended value for initiating highly active antiretroviral therapy (HAART). In this study, we investigated mRNA transcriptomes and miRNA profiles in PBMCs from this cohort of RPs at approximately 120 days postinfection. In comparison to samples from chronic progressors (CPs), whose clinical outcomes fall between the clinical outcome extremes of RPs and long-term nonprogressors (20), we identified a distinct cluster of genes expressed in RP PBMCs that is primarily associated with cell survival and apoptosis. We also determined a 5-miRNA signature characteristic of disease progression. Interestingly, the predicted targets of these miRNAs also converged in proapoptotic pathways.

Materials and Methods

STUDY POPULATION
Forty-six treatment-naive patients with EHI and 4 healthy controls (HCs) were enrolled in this study. EHI was defined by documented HIV-1 acquisition within the previous 6 months (21). Patients with EHI were divided into 2 groups, RPs (CD4+ T-cell counts <350 cells/µL within 1 year of infection; a CD4+ T-cell count of <350 cells/µL was considered as the end point for follow-up) and CPs (CD4+ T-cell counts remained >500 cells/µL after 1 year of infection). Both mRNA and miRNA expression profiles in the PBMCs were detected at approximately 120 days of HIV infection. We performed mRNA microarray analysis on 6 RPs and 7 CPs. The initial 347 miRNA array was performed in 11 RPs, 6 CPs, and 4 corresponding matched HCs (training group); only the miRNAs differentially expressed between RPs and CPs (P < 0.05) in the training group were detected in a subsequent validation group (19 RPs vs 10 CPs). Table 1 summarizes relevant characteristics of these groups. Ethics approval for these studies was obtained from the local ethics review committee, and written informed consent for participation in the study was obtained from all study participants. PBMCs were obtained from HIV-1–infected individuals and HCs by Ficoll–Hypaque density gradient centrifugation, cryopreserved in fetal calf serum supplemented with 10% DMSO, and stored in liquid nitrogen within 8 h of collection.

NIMBLEGEN GENE CHIP MICROARRAY
We thawed PBMCs from 6 RPs and 7 CPs and performed NimbleGen Gene chip microarray analysis at CapitalBio Corporation (Beijing, China). In brief, PBMCs were sent there and total RNA was extracted using Trizol reagent (Invitrogen). Array hybridization, washing, and scanning were carried out according to the NimbleGen’s Expression user’s guide. Comparisons of mRNA array data and principal component analysis (PCA) were performed with the Partek Genomics Suite software. Relative signal intensities for each gene were generated by using the robust multiarray average algorithm, and genes were determined to be significantly differentially expressed with a Benjamini–Hochberg false discovery rate (FDR)-adjusted P value <0.05. All microarray results have been deposited in the Gene Expression Omnibus database (accession no. GSE44216).

miRNA ARRAY ANALYSIS
Total RNA of PBMCs was isolated with Trizol reagent (Invitrogen). Escherichia coli polyA polymerase was used to add adenines at the 3’ end of RNA molecules lacking a polyA tail. After oligoT annealing, a universal tag was attached to the 3’ end of cDNAs during cDNA synthesis using retrotranscriptase Superscript III (Invitrogen). With this universal tag, an SYBR-based real-time quantitative PCR method was performed with miRNA–specific forward primers and a reverse universal primer mix. U1 and U6 were used in the training cohort and RNY3 and U6 were used in the validation cohort for normalization. The variation of changes in the threshold cycle (ΔCT) (CT, target-CT, and control) was evaluated and used as a relative qualitative value for further assay (22). Preprocessing of raw data and statistical analyses were performed using RealTime StatMiner software. Microarray raw data is available through the Gene Expression Omnibus repository (accession no GSE44332).

TARGET PREDICTION AND PATHWAY ENRICHMENT ANALYSIS
A list of genes with predicted binding sites for the selected miRNAs was downloaded from http://mirecords.biolead.org/. Only those genes that were predicted by at least 3 of the algorithms were selected. For pathway enrich-
ment analysis, genes differentially expressed in RPs and CPs and upregulated mRNAs in RPs which were the predicted targets of the miRNA candidates were input for pathway analysis with GeneGO Metacore software.

**miRNA OVEREXPRESSION AND CELL DEATH DETECTION**

A DNA sequence containing the pre-miRNA and flanking genomic sequence was amplified directly from genomic DNA, and the PCR-amplified fragments were cloned directly into an miRNA expression vector (pmaxGFP-mir). PBMCs were transfected by nucleofection with a pmaxGFP-miRNA vector or mock control with a T Cell Nucleofector kit (Lonza) and were incubated in a humidified 37 °C/5% CO₂ incubator for 24 h. Viable cells were then isolated by Ficoll–Paque density-gradient centrifugation and were plated in complete medium, either in the absence (unstimulated) or presence (stimulated) of soluble anti-CD3 and anti-CD28 (3 μg/mL each) (BD Biosciences) in 48-well plates. Spontaneous and activation-induced apoptosis were analyzed after 48 h of culture staining with the following fluorescence-labeled antibodies: allophycocyanin (APC)-anti-CD8, APC-Cy7-anti-CD28, PE (phycoerythrin)-Cy7-anti-CD4, Pacific blue annexin V, and 7–amino-actinomycin (AAD) (Biolegend). A FACSCanto II (BD Biosciences) was used for data acquisition and the data were analyzed with FlowJo software.

**DATA ANALYSIS**

SPSS 10.0 and prism 5 software were used to conduct statistical analyses. The relationships between miRNA, mRNA, and immune and viral factors were evaluated with Spearman correlation analysis. Paired t-tests were used for the comparison of expression levels of annexin V⁺ or 7–AAD⁺ between miR-31 and mock transfected cells. Mann–Whitney U-tests were used to assess differ-

### Table 1. Clinical characteristics of patients with EHI and HCs.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RPs</th>
<th>CPs</th>
<th>HCs</th>
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<td>6</td>
<td>4</td>
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<td>Han ethnic, no. (%)</td>
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<td>Subtype CRF01AE, no. (%)</td>
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<td>Age, mean (SD), years</td>
<td>36 (12)</td>
<td>30 (15)</td>
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<tr>
<td>Estimated days, mean (SD)</td>
<td>113 (17)</td>
<td>106 (16)</td>
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<td>CD4, cells/μL, mean (SD)</td>
<td>418 (165)</td>
<td>676 (92)</td>
<td>715 (113)</td>
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<td>Viral load, log copies/mL, mean (SD)</td>
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<td><strong>miRNA validation cohort</strong></td>
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<tr>
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<td>6 (67)</td>
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<td>114 (17)</td>
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<td>CD4, cells/μL, mean (SD)</td>
<td>288 (90)</td>
<td>661 (116)</td>
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<td>6 (86)</td>
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<td>8 (61.5)</td>
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<td>39 (9)</td>
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<tr>
<td>Estimated days, mean (SD)</td>
<td>114 (17)</td>
<td>115 (17)</td>
<td></td>
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<tr>
<td>CD4, cells/μL, mean (SD)</td>
<td>306 (120)</td>
<td>715 (134)</td>
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<tr>
<td>Viral load, log copies/mL, mean (SD)</td>
<td>4.33 (0.81)</td>
<td>3.61 (0.74)</td>
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ences of miRNAs. *P* values <0.05 were considered to be statistically significant. ROC analysis was performed to evaluate the predictive performance of the disease progression–related miRNA signatures. For ROC analysis of the 5-miRNA signature combination, *P* (probability of a patient sample) was calculated for inclusion in the ROC analysis by the formula: 

\[ X = \ln(P/1-P) = b_0 + b_1 \Delta CT_1 + b_2 \Delta CT_2 + b_3 \Delta CT_3 + \ldots + b_n \Delta CT_n, \]

where the b terms were the *i*th regression coefficients by binary logistic regression, and the \( \Delta CT_i \) terms were the relative expression levels of each miRNA. In this study, \( X = -26.822 - 3.087 \times \text{miR}-31 - 0.649 \times \text{miR}-200c + 0.855 \times \text{miR}-99a + 0.588 \times \text{miR}-503 - 0.342 \times \text{miR}-526a. P = e^{\Delta X}(1 + e^{\Delta X}) \).

Kaplan–Meier (K–M) techniques and multivariate least-square regression models were used to determine the effect of miRNA expression levels on time-dependent disease progression. CD4+ T-cell counts <350 cells/μL (the recommended CD4+ T-cell count for initiating HAART therapy) were considered as the end points in K–M analysis and regression models. The hazard ratio with 95% CIs was reported as an estimate of overall disease progression risk in multivariate least-square regression models.

**Results**

**A DISTINCT TRANSCRIPTOME BETWEEN HIV-INFECTED RPs AND CPs**

With unsupervised PCA of all transcript data from the microarray, we observed that 6 RPs and 7 CPs segregated into groups (Fig. 1A). Of the 45 034 transcripts examined, 935 were differentially expressed between RPs and CPs (FDR-controlled *P* < 0.05) (Fig. 1B), including 605 upregulated and 330 downregulated genes in RPs. Among these genes, 391 upregulated and 286 downregulated genes had a fold change of over 50%. Pathway enrichment analysis showed that the 391 upregulated genes in RPs are primarily involved in pathways associated with apoptosis, development, cell cycle, and DNA damage (Fig. 1C; also see Table 1 in the Data Supplement that accompanies the online version of this report at http://www.clinchem.org/content/vol59/issue8). Three apoptosis-associated genes [apoptotic peptidase activating factor 1 (APAF1), mitogen-activated protein kinase kinase 4 (MAP2K4), and tumor protein p53 (TP53)] involved in the p53-dependent apoptosis pathway were upregulated in PBMCs from RPs. Importantly, APAF1 and MAP2K4, together with 2 other upregulated genes, deleted in liver cancer 1 (DLC1) and N-myristoyltransferase 1 (NMT1), participate in the cytoplasmic/mitochondrial transport of the proapoptotic proteins Bid, Bmf, and Bim. Moreover, APAF1 and MAP2K4, together with another upregulated gene, eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa (EIF2S1), are involved in the endoplasmic reticulum stress response apoptotic pathway.

We assessed the relevance of the 6 upregulated genes in the apoptosis pathways with disease progression by K–M survival and ROC analysis. We found that the increased expression of each gene can reliably predict rapid disease progression (see online Supplemental Fig. 1). These results suggest that multiple apoptosis pathways are activated in PBMCs from RPs.

**A 5-miRNA SIGNATURE DISTINGUISHES RPs FROM CPs**

Previous studies have found that most miRNAs with altered expression were downregulated in chronic HIV-infected patients (23–24). However, little is known about the miRNA profiles of PBMCs in RPs, especially during EHI. With 17 early HIV-infected patients (including 11 RPs and 6 CPs) and 4 age- and sex-matched HCs, we formed a training cohort and quantified expression levels of 347 miRNAs using real-time quantitative PCR-based arrays. Unsupervised hierarchical clustering successfully distinguished most of the patients with EHI from HCs (Fig. 2A). In patient PBMCs compared to those of HCs, miRNAs were significantly downregulated, whereas 13 miRNAs were significantly upregulated at the EHI stage (*P* < 0.05) (Fig. 2B; also see online Supplemental Table 2A). Some downregulated miRNAs were reported to have anti-HIV effects, such as the miR–17–92 cluster (miR–19a/19b), which inhibits HIV replication (25).

We continued to refine an miRNA signature associated with rapid disease progression. Because the viral set point is a key determinant of clinical outcome, we examined whether miRNAs associated with rapid disease progression were also correlated with viral set point by dividing the patients with EHI into 2 groups, patients with high (>4.5 log copies/mL) or low (<4 log copies/mL) viral set points (Fig. 3A). The viral set point was calculated by averaging all viral load measurements between 120 days and 12 months after the determined date of infection. In the training cohort, we identified a total of 22 differentially expressed miRNAs in RPs and CPs (*P* < 0.05) (Fig. 3B; also see online Supplemental Table 2B). Among them, 14 miRNAs were downregulated and 8 were upregulated (*P* < 0.05) (Fig. 3B; also see online Supplemental Table 2B) in RPs. Meanwhile, 12 miRNAs were significantly downregulated in patients with a high set point (*P* < 0.05, Fig. 3B). From a
total of 12 miRNAs, 4 (miR-31, miR-99a, miR-200c, and miR-126–5p) overlapped with the miRNAs identified in the RP vs CP analysis (Fig. 3B). Twenty-two differentially expressed miRNAs identified in the comparison of RPs vs CPs in the training group were subjected to validation with samples from another enlarged cohort (19 RPs and 10 CPs). After the validation, we determined that 5 miRNAs, including miR-31, miR-200c, miR-99a, miR-503, and miR-526a, were consistently suppressed in the PBMCs of RPs, with a fold change ranging from 2.10 to 52.21 (Fig. 3C; also see online Supplemental Table 2B). With complete
The linkage used in the unsupervised clustering method, we distinguished most of the RPs from CPs with these 5 miRNAs (Fig. 3D).

The 5-miRNA signature is associated with disease progression

We next examined whether this 5-miRNA signature segregating RPs from CPs was related to disease progression. The expression level of each individual miRNA correlated positively with patients’ CD4⁺ T-cell counts, and the expression levels of miR-31 and miR-200c correlated negatively with viral load or with the viral set point (see online Supplemental Fig. 2). We then analyzed the predictive power of this 5-miRNA signature for disease progression in the validation cohort. As determined by ROC analysis, the 5-miRNA signature had a predictive accuracy of 94% as measured by the area under the curve (AUC) for rapid disease progression (Fig. 3E). Of the 5 miRNAs, 4 (miR-31, miR-200c, miR-526a, and miR-99a) were predictive of rapid disease progression with significant accuracy individually (Fig. 3E). In a survival analysis, lower expression of miR-31, miR-200c, and miR-526a was highly predictive of disease progression (Fig. 3F). When the expression levels of miR-31, miR-200c, and miR-526a were included in a multivariate Cox regression model with both CD4⁺ T cells and viral load, lower expression of miR-31 independently predicted rapid disease progression, which highlighted its value in prognosis during EHI (see online Supplemental Table 3).

The impacts of signature miRNAs converge on the apoptosis pathway

It was estimated that >60% of cellular mRNA transcripts are regulated by miRNAs (26). We investigated whether these 5 signature miRNAs contributed to the altered mRNA profiles observed in RPs. We developed a gene target list using miRecords (27), and only those genes that were predicted by at least 3 of the algorithms were selected (see online Supplemental Table 4). Because all of these 5 miRNAs in PBMCs from RPs were downregulated, we directly assessed their impact on
Fig. 3. Differential miRNA expression between RPs and CPs.

(A), Grouping of EHI patients. Patients with EHI were divided into RPs (CD4⁺ T-cell counts <350 cells/μL within 1 year of infection) and CPs (CD4⁺ T-cell counts remained >500 cells/μL after 1 year of infection). The CD4⁺ T-cell number represents the first CD4⁺ T-cell number less than 350 cells/μL from 120 days to 1 year of infection for RPs or the first CD4⁺ T-cell number after 1 year of infection for CPs. On the basis of viral set point, patients with EHI were divided into groups with high (>4.5 log copies/mL) or low (<4 log copies/mL) viral set points. (B), miRNAs differed significantly (P < 0.05) in patients with different rates of disease progression or different viral set points in the training cohort (left, RP vs CP; right, high vs low viral loads). Upregulated miRNAs were in blue and downregulated miRNAs were in black. Overlapping area indicates miRNAs significantly downregulated in both RPs and patients with high viral set points compared with CPs or patients with low set points. (C), miRNAs that differed significantly in the comparison of RPs and CPs in the training cohort were detected in a subsequent validation cohort. Five miRNAs were validated and fold changes of the 5 miRNAs in both the training and validation cohorts are shown (P < 0.05). (D), Hierarchical clustering of the 5 miRNAs differentially expressed between RPs and CPs in the validation cohort by the complete linkage method and Pearson correlation. (E), The predictive value of the 5-miRNA signature combination or individual miRNAs for rapid disease progression was calculated by ROC analysis. The AUC was used to evaluate disease progression predictions. (F), According to the median value of miRNA (ΔCT) expression, EHI patients in the validation cohort were divided into high expressers (above the median value) and low expressers (below the median value). CD4⁺ T-cell counts reaching <350 cells/μL were considered as the end point for follow-up. K–M survival analysis showed that the mean time for CD4⁺ T-cell counts to reach <350 cells/μL in low expressers (miR-31, miR-200c, and miR-526a) was significantly shorter than that in high expressers (P < 0.05).
the significantly upregulated genes identified in our mRNA microarray experiments.

Within the 391 genes upregulated in RP PBMCs with a fold change of >50%, 129 genes were predicted targets of at least 1 of the 5 miRNAs (Fig. 4A; also see online Supplemental Table 5). Our bioinformatics analysis indicated that each miRNA preferentially impacted different pathways in RP PBMCs (see online Supplemental Table 6). Specifically, the impact of miR-31 was largely enriched in apoptosis and DNA damage responses. We next pooled together 5 groups of upregulated predicted targets of each signature miRNA with the 391 upregulated genes to perform pathway enrichment analysis. In this combined analysis, the impact of the 5-miRNA signature converged into the pathway of apoptosis and survival: cytoplasmic/mitochondrial transport of the proapoptotic proteins Bid, Bmf, and Bim (Fig. 4B). Within this pathway, 4 genes were significantly upregulated in RPs and 3 of them were the predicted targets of the 5 miRNAs (APAF1, MAP2K4, and NMT1) (Fig. 4C). This suggests that mRNA expression and the predicted signature miRNA targets converged to the regulation of cytoplasmic/mitochondrial transport of proapoptotic BH3-only family proteins in RP PBMCs.

**Fig. 4. Combined analysis with mRNA expression and predicted signature miRNA targets demonstrated converged enrichment to the apoptosis pathway.**

(A), In the 391 significantly upregulated genes with a fold change of >50% within the RP transcriptome (P < 0.05), 129 were the predicted targets of signature miRNAs. All upregulated predicted targets (from 5 miRNAs) with the 391 upregulated genes were put into pathway enrichment analysis. (B), By GeneGO pathway enrichment analysis, the upregulated mRNA expression and predicted signature miRNA targets demonstrated converged enrichment solely to the pathway of apoptosis and survival: cytoplasmic/mitochondrial transport of the proapoptotic proteins Bid, Bmf, and Bim. (C), Four genes were involved in this pathway and 3 of them were the predicted targets of the 5 miRNAs. The x axis represents the significantly upregulated genes involved in the pathway. The y axis represents the individual miRNAs. The z axis represents the fold change of the genes in the comparison of RPs with CPs. And for genes with multiple probes in the microarray, the mean value was calculated.

miR-31 PROMOTES T CELL SURVIVAL

The above bioinformatic analysis suggested that the reduced expression of these 5 miRNAs may directly result in increased death of CD4+ T cells and rapid disease progression. Reciprocal to our observation, the expression level of miR-31 is highly increased in HIV elite controllers (18). However, the function of miR-31 in lymphocyte survival is unknown. We tested whether the expression of miR-31 enhanced the survival of human primary T cells. We transiently transfected plasmids expressing GFP alone (mock control) or GFP plus miR-31 into primary human PBMCs by electroporation. GFP+ cells were further cultured to monitor the rate of apoptosis. Enforced expression of miR-31 readily protected both CD4+ (Fig. 5, A and B) and CD8+ T cells (Fig. 5, C and D) from spontaneous and activation-induced cell death (P < 0.01). Interestingly, this protection was more striking at the later stage of cell death (7AAD+ cells, Fig. 5, B and D, lower panels), which indicated that miR-31 either prohibits the death of annexin V+ T cells or inhibits nonapoptotic cell death.

Discussion

Focusing on the earliest events during AIDS progression, we set up an open prospective cohort, and over
the past 4 years, enrolled more than 2000 high-risk study participants. Most participants were HIV-negative men who have sex with men, a population with alarming HIV prevalence in China (28). In this study, 46 HIV-infected patients with well-documented dates of infection were identified. Among them, 30 patients experienced a rapid loss of CD4+ T cells, reaching a count of <350 cells/μL blood within 1 year of

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**Fig. 5. miR-31 overexpression promotes T-cell survival.**

Primary PBMCs transfected by nucleofection with pmaxGFP-miR-31 or mock vector (Mock) were either unstimulated or stimulated with anti-CD3 and anti-CD28 (+αCD3αCD28) (3 μg/mL each). The proportion of annexin V+ cells was detected 48 h after stimulation and compared by paired t-test. (A), Representative data of CD4+ T-cell death ex vivo either with no stimulation or after T-cell receptor stimulation. The frequencies of cells are indicated as the percentage of total CD4+ GFP+ T cells. (B), The proportion of annexin V+ cells or 7-AAD+ cells of CD4+ T cells was significantly decreased by miR-31 overexpression compared with mock (P < 0.05) in either unstimulated or anti-CD3/anti-CD28 stimulated CD4+ T cells. (C), Representative data of CD8+ T-cell death ex vivo either with no stimulation or after T-cell receptor stimulation. The frequencies of cells are indicated as percentage of total CD8+ GFP+ T cells. (D), The proportion of annexin V+ cells or 7-AAD+ cells of CD8+ T cells was significantly decreased by miR-31 overexpression compared with mock (P < 0.05) in either unstimulated or anti-CD3/anti-CD28 stimulated CD4+ T cells; (n = 6).
infection. Extensive efforts over the past decades have explored mechanisms associated with early AIDS progression (29), but the etiology underlying such rapid progression remains poorly understood. Early emergence of CXCR4-utilizing HIV strains was found to accelerate disease progression (5). We have isolated primary HIV from CD4+ T cells of 15 EHI patients enrolled in this study (13 RPs, 2 CPs). Tropism analysis showed that all the viruses isolated from RPs were CCR5 utilizing but not CXCR4 utilizing (unpublished data), indicating that viral tropism was not the cause for the rapid disease progression in the RPs enrolled in our study. In addition, we performed an SNP analysis of interleukin-7RA, TLR9, and LEDGF (lens epithelium-derived growth factor) on RPs. The results showed that there were risk alleles in some but not all of the RPs (see online Supplemental Table 7), suggesting that the SNPs alone do not explain the rapid disease progression observed in our study. CPs in our cohort had viral and immunologic backgrounds similar to those of RPs, such as the HIV subtype, ethnicity, sex, and transmission route. This provides a unique opportunity for us to probe the mechanisms that elicit variable AIDS progression during EHI.

Compared to CPs, the prominent characteristic in the transcriptome of RPs is the increased expression of proapoptotic genes; accordingly, the signature change in their miRNA profile demonstrates lowered expression of 5 miRNAs that suppress the expression of cell death–related genes. This result is not entirely surprising: in vitro HIV infection rapidly activated the expression of proapoptotic genes in CD4+ T cells (30); programmed cell death upon HIV infection and bystander activation were the major cause of CD4+ T cell destruction (9); and CD4+ T cells from long-term nonprogressors are significantly protected from spontaneous apoptosis (31). Our study indicates that, in addition to CD4+ T cells, the whole PBMC population has been reprogrammed to accelerate the loss of cells in RPs. This is consistent with our previous observation that plasmacytoid dendritic cells were rapidly diminished in RPs (32). It has also been reported that the accelerated apoptosis in HIV-infected lymph nodes affects all functional compartments of the lymph node, including CD8+ T cells and B cells (33). We postulate that bystander activation during the earliest anti-HIV response might be the primary cause for the shift of the RP PBMC transcriptome to favor cell death. Collectively, the combined early loss of anti-HIV immunity and rapid death of CD4+ T cells may contribute to rapid AIDS progression. It should be noted that our PBMC samples were cryopreserved because it is impractical to study freshly isolated samples in this cohort. Cryopreservation may trigger some apoptotic pathways; however, because the PBMCs from RPs, CPs, and HCs were all cryopreserved, the cryopreservation-caused changes in these samples were normalized.

Another 2 significant changes in the transcriptomes of RPs are related to genes regulating the cell cycle and DNA damage. The interplay between the cell cycle and HIV infection is complex. On the one hand, in vitro viral infection arrested cell cycles in CD4+ T-cell lines (34–35); on the other hand, the upregulation of genes involved in the cell cycle was correlated positively with the HIV viral load (13), suggesting that the accelerated cell cycle was exploited for viral amplification (36). The transcriptome changes we observed indicate an enhanced cell cycle progression in the immune compartment of RPs. This could also accelerate immune cell loss through hyperactivation and increased turnover (17, 36). In addition, HIV or HIV accessory proteins activate the DNA damage response (37–38). If the DNA damage is not repaired or tolerated, cells that harbor DNA damage will be removed from the population by death or senescence (39–40). Taken together, the transcriptome and miRNA profiles in RPs reflect that the loss of protection against cell death might be the pathological cause of their disease progression.

In this study, we identified a 5-miRNA signature distinguishing RPs from CPs, which could serve as a potential biomarker for the prediction of disease progression in EHI. Among these miRNAs, miR-31 alone had a predictive accuracy of 93% and independently predicted fast disease progression in a multivariate Cox regression model, suggesting the significance of utilizing miR-31 as a biomarker candidate for predicting rapid disease progression. We postulate that lower expression of miR-31 could contribute to fast disease progression through decreased degradation of the potential mRNAs in the apoptosis pathway. Consistent with this result, our functional study demonstrates that enhanced expression of miR-31 promotes primary T-cell survival. Taken together, miR-31 is underscored both in the identification of biomarker candidates and in the determination of courses for rapid disease progression.

Identification of biomarkers to distinguish RPs from CPs is critical for early clinical intervention. It is technically easier to isolate PBMCs than individual cell subtypes. Thus, PBMCs serve as practical and accessible samples for the clinical detection of biomarkers. Owing to the difficulties in recruiting patients with EHI, our cohort size is relatively modest. Further longitudinal studies are needed to verify the identified signature of 5 miRNAs as biomarkers for the prediction of HIV disease progression. In conclusion, our study shows for the first time the mRNA and miRNA transcriptomic profile of patients who become HIV RPs.
and suggests that deregulated gene pathways and miRNA signatures are tightly associated with fast disease progression. Our results provide novel insights to HIV rapid disease progression.

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