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Biochemical and Biophysical Research Communications 349 (2006) 59–68
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# Human microRNA clusters: Genomic organization and expression profile in leukemia cell lines

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Received 19 July 2006
Available online 11 August 2006

**Abstract**

MicroRNAs (miRNAs) play an important role in diverse physiological and developmental processes by negatively regulating expression of target genes at the post-transcriptional level. Here, we globally analyzed the genomic organization of all registered 326 human miRNA genes in miRNA registry 7.1 and found that 148 human miRNA genes appeared in a total of 51 clusters. Alignment of the miRNA sequences in different clusters revealed a significant number of miRNA paralogs among the clusters, implying an evolution process targeting the potentially conserved roles of these molecules. Then we performed Northern blot analysis for expression profiling of all clustered miRNAs in several human leukemia cell lines. Consistent expression of the miRNAs in a single cluster was revealed in 39 clusters, while inconsistent expression of members in a single cluster was detected in the other 12 clusters. Meanwhile, we identified several hematopoietic lineage-specific or -enriched miRNA clusters (e.g., the mir-29c, mir-302, mir-98, mir-29a, and let-7a-1 clusters) and individual miRNAs (e.g., mir-181c, mir-181d, mir-191, and mir-136). These findings may suggest vital roles of these miRNA clusters or miRNAs in human hematopoiesis and oncogenesis, and provide clues for understanding the function and mechanism of miRNAs in various biological processes.


*Keywords:* MicroRNA clusters; Human leukemia cell lines; Northern blot; Genomic organization

MicroRNAs (miRNAs) are a novel class of conserved 21–23 nucleotides long RNAs with important roles in regulating gene expression [1]. They are generated from intergenic or intronic transcripts that are known as primary transcripts (pri-miRNAs). The pri-miRNAs are first cleaved by the RNase III enzyme Drosha into precursors that are 70 nucleotide-long and have hairpin structures (pre-miRNAs) in the nucleus [2,3]. These pre-miRNAs, are then exported into the cytoplasm by the nuclear export factor Exportin-5, and finally processed into mature miRNAs by another RNase III enzyme, Dicer [4,5]. Mature miRNAs are incorporated into the RISC/miRNP complexes and target specific mRNAs to trigger either mRNA degradation, which is the dominant process in plants whose miRNAs exhibit a near-perfect match with their targets, or translation repression, which is dominant in animals whose miRNAs generally show a lower degree of complementarity to their targets [6].

miRNAs have diverse biological functions in developmental and physiological processes, but so far, only a handful of miRNAs have been carefully studied, for example, mir-196 is involved in HOX gene regulation [7]; mir-375 regulates the Myotrophin (Mtpn) gene, and thereby glucose-stimulated insulin exocytosis [8]; mir-1 regulates the balance between differentiation and proliferation of cardiomyocytes during heart development in mice [9]; mir-181, -142, and -223 modulate hematopoietic lineage differentiation in mice [10]; mir-143 regulates human adipocyte differentiation [11]; mir-122a may have a role in the exclusion of the cationic amino acid transporter (CAT-1)

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0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.07.207

protein from the liver [12]. As these studies suggest, efforts principally focus on single miRNAs and are still incomplete.

Recently, following the discovery of miRNA gene clusters, some groups indicated that miRNAs might work in combination to accomplish their function throughout many biological processes. For example, expression of the mir-143 cluster is down-regulated in colon cancer as well as in some other cancers cell lines [13]. The mir-430 cluster regulates neurogenesis in zebrafish [14]; The mir-17 cluster modulates E2F1 expression and might be a potential human oncogene [15]; and the mir-15a cluster can induce leukemia cell apoptosis by targeting BCL2 [16]. Remarkably, as demonstrated by a recent study, the proportion of clustered miRNAs in humans goes far beyond what was previously envisioned [17]. Generally, it is thought that miRNA genes disperse in the genome in an intergenic or intronic manner and are usually transcribed by RNA polymerase II [18]. Previous studies found that a substantial fraction of miRNAs is located on polycistronic transcripts, implying a common phenomenon of miRNA clustering [19]. This natural genomic organization pattern of miRNA genes provides internal mechanisms for them to function in coordination. Thus, it is reasonable to speculate that these human clustered miRNAs might constitute a complicated regulatory network to function by targeting more or fewer special mRNAs. Clustered miRNAs are generally similar in sequence but can differ, and a cluster usually includes two or three miRNA genes. But larger clusters composed of more miRNA genes have also been identified, including a human mir-17 cluster comprising 6 miRNA genes and a human mir-302 cluster comprising 8 miRNA genes.

Mammalian blood cells consist of at least eight distinct cell lineages, all of which are derived from a common precursor called the hematopoietic stem cell (HSC) [20]. The accessibility and interest of the hematopoietic system have made it one of the most suitable models for understanding mammalian development and cell differentiation. Hematopoiesis is a multiply regulated process whose overall spatial and temporal organization ensures the normal formation of all mature blood cells. This complicated differentiation program generally depends on the incorporation of a number of regulators rather than a single one. This view highlighting the synergic function of molecules has been confirmed by the interactions of diverse transcription factors involved in hematopoietic cell fate determination in mammals [21]. Moreover, several miRNAs whose genes dispersed on genome have been proved to participate in hematopoiesis [22]. Thus, we suspect that some miRNAs located at a single cluster containing more than one similar or different member may together contribute to mammalian hematopoiesis.

In this study, we first analyzed the clustering properties of all 326 human miRNA genes from the miRNA registry release edition 7.1 and found that 148 miRNA genes are organized in a total of 51 clusters. Then we performed detailed homologous analysis among miRNAs in a single cluster and among the miRNA clusters by sequence alignment, identifying nine paralogous groups. Subsequent Northern blot analysis in diverse hematopoietic cell lines showed consistent expression of the miRNA members in the identical cluster in 78% of human miRNA clusters, while there was inconsistent expression in almost one-quarter of them. Notably, we identified several hematopoietic lineage-specific or -enriched miRNA clusters, implying involvement of miRNA clusters in hematopoiesis and leukemia. This work is the first systematic report of hematopoietic expression profiling of human miRNA clusters and provides new clues for understanding the function and mechanism of miRNA clusters in diverse biological processes.

## Materials and methods

*Genomic analysis of human miRNA clusters.* The human miRNAs analyzed in this study (326 in total) were derived from the miRNA registry release 7.1 (http://www.sanger.ac.uk//software/Rfam/mirna/). The genomic locations were derived from the UCSC network resources (http://genome.ucsc.edu). All 326 human miRNA genes were used for the general clustering analysis. We evaluated and extracted the general clustering of miRNA genes according to the method of Altuvia et al. [17] and analyzed the clustering region of miRNAs using the following RNA polymerase II promoter analysis software.

Promoter Scan (http://thr.cit.nih.gov/molbio/proscan) for predicting promoter regions based on scoring homologies with putative eukaryotic Pol II promoter sequences, Promoter Prediction 2.0 (http://www.cbs.dtu.dk/services/Promoter/) for predicting transcription start sites of vertebrate Pol II promoters in DNA sequences, and NNPP (neural network promoter prediction) (http://www.fruitfly.org/seq_tools/promoter.html) for predicting eukaryotic Pol II promoter sequences. Then the miRNA clusters in accordance with our criteria as described above were selected for subsequent experiments.

*Homology analysis of human miRNA clusters.* We systematically analyzed homologous genes of human clustered miRNAs and identified miRNA clusters that can form paralogs using the following software: sequence alignment of mature miRNAs and precursor-miRNAs were obtained by clustalW and Genedoc.

*Leukemia cell lines and preparation of total RNA.* The following human leukemia cell lines, which represent to a certain extent the main hematopoietic lineage lines, were used in this study. They are: K562 (chronic myelogenous leukemia cell line), HEL (human erythroleukemia cell line), HL-60 (human promyelocytic leukemia cell line), Jurkat (human acute T cell leukemia cell line), HUT-78 (human T lymphoma cell line), CMK (human megakaryoblastic leukemia cell line), 3D5 (human B cell line), Raji (human B-non-Hodgkin's lymphoma cell line), U937 (human promonocytic leukemia cell line), and THP-1 (human promyelocytic leukemia cell line). A non-hematopoietic cell line, HeLa cells (cervical adenocarcinoma), was also used as a control. All the cells were grown in RPMI 1640 (Gibco/BRL) supplemented with 10% FBS (Clontech) at 37 °C in 5% $CO_2$. Total RNA was extracted from all the cell lines with Trizol reagent (Invitrogen) following the manufacturer's instructions.

*Expression detection of miRNA clusters.* Northern blot analysis was performed as described previously with minor modifications [10]. Total RNA was denatured at 55 °C for 30 min with deionized formamide. A total of 30 μg per lane was loaded onto a 15% polyacrylamide TBE gel and separated using 0.5 × TBE as a running buffer at 14 mA for 3 h. After electrophoresis, the RNA was transferred to a Hybond-$N^+$ membrane (Amersham) at 200 mA for 2 h by an electro-transferring system, and then it was crosslinked with ultraviolet radiation for 150 s, followed by baking it at 80 °C for 1 h. Oligonucleotides complementary to the corresponding

mature miRNA sequences were synthesized and used for the probes. Twenty nanometers of each probe was prepared by T4 polynucleotide kinase with $\gamma$-$^{32}$P dATP. Blots were pre-hybridized for 2 h at 37 °C using hybridization buffer (BioDev) in hybridization tubes. Subsequently $\gamma$-$^{32}$P-end-labeled miRNA probes were added into tubes and incubated for 16 h at 37 °C. After hybridization, the membranes were washed twice for 5 min using buffer 1 (0.1% SDS, 1 × SSC) at room temperature and for 10 min using buffer 2 (0.1% SDS, 0.5 × SSC) at 37 °C and then exposed to X-ray films for 72 h at −70 °C. U6snRNA was used as a probe for checking equal RNA loading. Then the membranes were analyzed using a Fujifilm-BAS2500 phosphorimager and signal intensity was quantitated using Analytical Imaging Station (version 3.0) software.

*Data analysis.* Northern blot images of each cluster were normalized to the U6 snRNA and included for comparison of miRNA levels respectively. The miRNA numbers that showed positive signal were counted. The expression pattern of miRNAs was compared to their gene organization in chromosomes, and the miRNAs of hematopoietic specific-lineage or -enriched expression were also stepwise classified.

*Validation of expression and correlation of miRNA clusters.* In hybridization experiments, we first selected 6 cell lines that could represent 6 main hematopoietic lineage lines and initially filtered clustering miRNAs that showed lineage-specific or -enriched expression. Then we used 11 cell lines to broaden and deepen the investigation. More validations were employed in clustering miRNAs that appeared to be of paralogous composition and to exhibit lineage-specific or -enriched expression.

## Results and discussion

### *Genomic organization of human miRNA clusters*

To date, there are a total of 326 registered human miRNA genes in RNA registry 7.1. They are either experimentally identified or computationally predicted homologues of other mammalian miRNA genes. In a recent study, performed on a dataset of 207 human miRNAs, 31 miRNA clusters were isolated [17]. Here, according to Altuvia's strategy for defining a miRNA cluster, we analyzed all of the 326 human miRNA genes.

Because it may be too arbitrary to define the region of miRNA clusters in terms of their genomic location, we subsequently analyzed their promoter regions to refine the primary identification of a cluster. We used three distinct programs to analyze and predict the potential transcription start site and promoter region of each intergenic miRNA cluster, and selected the highest score for comparison. In nearly all miRNA clusters except for mir-127 and mir-521-1 cluster, no putative promoters were identified in the regions between pre-miRNAs within a single cluster. While other miRNA clusters, which were located at the introns or UTRs of protein-coding genes, were considered to transcribe together with their host genes [23]. The results of promoter analysis for intergenic miRNAs are summarized in supplemental Table 1 (Table S1). Through the above bioinformatics analysis, we finally identified 148 miRNAs organized into 51 clusters.

We then checked the sequences of individual miRNAs among these clusters and noted that many miRNA paralogs with one or more nucleotides substitutions existed not only in a single miRNA cluster but also among different miRNA clusters. Thus, we performed sequence alignment using the ClustalW software to detect the conservation patterns (data not shown). We finally identified 38 human miRNA clusters containing homologous miRNAs (Table 1) and 26 clusters forming 9 distinct paralogous cluster groups (Table 2). Fig. 1 shows the sequence alignment of homologous miRNAs in 6 paralogous clusters. The alignment results revealed that the 5′ ends of these paralogous sequences showed higher conservation; residues 2–8 especially were nearly perfectly conserved. This finding may also confirm published reports that miRNAs might negatively regulate translation of specific target mRNAs by complementarily pairing of their 2-8 core sites to bases in the 3′ UTR of targets [24,25]. Remarkably, nearly all members of each cluster in three paralogous groups contain nucleic acid sequences highly homologous with each other (Paralogs 1–3, Table 2). Moreover, they were partial to appear in pairs while distributing in different

Table 1
Homology analysis of Human miRNA clusters

| Cluster | Members[a] | Homologs[b] | Paralogous clusters[c] |
|---|---|---|---|
| mir-200b | 3 | 3 | 1 |
| mir-181b-1 | 2 | 2 | 2 |
| mir-29c | 2 | 2 | 1 |
| mir-30e | 2 | 2 | 0 |
| mir-15b | 2 | 2 | 1 |
| mir-302 | 5 | 4 | 0 |
| mir-29a | 2 | 2 | 1 |
| mir-25 | 3 | 2 | 2 |
| let-7a-1 | 2 | 2 | 2 |
| mir-23b | 3 | 2 | 1 |
| mir-181a | 2 | 2 | 2 |
| mir-34b | 2 | 2 | 0 |
| mir-200c | 2 | 2 | 1 |
| mir-17 | 6 | 3 | 2 |
| mir-16 | 2 | 2 | 1 |
| mir-379 | 4 | 3 | 0 |
| mir-368 | 2 | 2 | 0 |
| mir-132 | 2 | 2 | 0 |
| mir-23a | 3 | 2 | 1 |
| mir-99b | 3 | 0 | 1 |
| mir-181c | 2 | 2 | 2 |
| mir-371 | 3 | 3 | 0 |
| mir-518e | 3 | 2 | 6 |
| mir-518d | 6 | 3 | 6 |
| mir-521-1 | 5 | 3 | 6 |
| mir-515-1 | 3 | 3 | 6 |
| mir-526b | 7 | 5 | 6 |
| mir-526a-1 | 6 | 5 | 6 |
| mir-520d | 3 | 2 | 6 |
| mir-99a | 2 | 0 | 1 |
| let-7a-3 | 2 | 2 | 2 |
| mir-221 | 2 | 2 | 0 |
| mir-98 | 2 | 2 | 2 |
| mir-363 | 4 | 2 | 2 |
| mir-105 | 2 | 2 | 0 |
| mir-450 | 2 | 2 | 0 |
| mir-500 | 3 | 2 | 0 |
| mir-514 | 3 | 3 | 0 |

[a] The number of miRNAs in each of human miRNA clusters.
[b] The number of homologous miRNAs in each of human miRNA clusters.
[c] The number of paralogous clusters that are involved in each of human miRNA clusters.

Table 2
Paralogous groups of human miRNA clusters

| Paralogs | Clusters | miRNAs | Homologous type |
|---|---|---|---|
| 1 | let-7a-1-cluster | let-7a-1; let-7f-1 | HH[a] |
| | let-7a-3-cluster | let-7a-3; let-7b | HH |
| | 98-cluster | hsa-mir-98; let-7f-2 | HH |
| 2 | 29c-cluster | hsa-mir-29c; hsa-mir-29b-2 | HH |
| | 29a-cluster | hsa-mir-29a; hsa-mir-29b-1 | HH |
| 3 | 15b-cluster | hsa-mir-15b; hsa-mir-16-2 | HH |
| | 16-1-cluster | hsa-mir-16-1; hsa-mir-15a | HH |
| 4 | 181b-1-cluster | hsa-mir-181b-1; hsa-mir-213 | PH[b] |
| | 181a-cluster | hsa-mir-181a; hsa-mir-181b-2 | PH |
| | 181c-cluster | hsa-mir-181c; hsa-mir-181d | PH |
| 5 | 25-cluster | hsa-mir-25; hsa-mir-93; hsa-mir-106b | PH |
| | 17-cluster | hsa-mir-17; hsa-mir-18; hsa-mir-19a; hsa-mir-20; hsa-mir-19b-1; hsa-mir-92-1 | PH |
| | 106a-cluster | hsa-mir-363; hsa-mir-92-2; hsa-mir-19b-2; hsa-mir-106a | PH |
| 6 | 99b-cluster | hsa-mir-99b; hsa-mir-125a; let-7e | PH |
| | 99a-cluster | hsa-mir-99a; let-7c | PH |
| 7 | 23b-cluster | hsa-mir-23b; hsa-mir-27b; hsa-mir-24-1 | PH |
| | 23a-cluster | hsa-mir-24-2; hsa-mir-23a; hsa-mir-27a | PH |
| 8 | 200b-cluster | hsa-mir-200b; hsa-mir-200a; hsa-mir-429 | PH |
| | 200c-cluster | hsa-mir-200c; hsa-mir-141 | PH |
| 9 | 518e-cluster | hsa-mir-518e; hsa-mir-526c; hsa-mir-518a-1 | PH |
| | 518d-cluster | hsa-mir-518d; hsa-mir-526a; hsa-mir-516-4; hsa-mir-518a-2; hsa-mir-517c; hsa-mir-520h | PH |
| | 521-1-cluster | hsa-mir-521-1; hsa-mir-526c; hsa-mir-522; hsa-mir-519a-1; hsa-mir-527; hsa-mir-516-1 | PH |
| | 515-1-cluster | hsa-mir-515-1; hsa-mir-519e; hsa-mir-520f | PH |
| | 526b-cluster | hsa-mir-526b; mir519b;hsa-mir-525; mir523; hsa-mir-526c; hsa-mir-518f; hsa-mir-520b; hsa-mir-518b | PH |
| | 526a-1-cluster | hsa-mir-526a-1; hsa-mir-520c; hsa-mir-518c; hsa-mir-524; hsa-mir-517a; hsa-mir-519d | PH |
| | 520d-cluster | hsa-mir-520d; hsa-mir-517b; hsa-mir-520g | PH |

[a] Highly homologous (HH) cluster: sequence homology of all miRNAs belonging to a paralogous group exceeds 90%.
[b] Partially homologous (PH) cluster: sequence homology of miRNAs belonging to a paralogous group is between 50% and 90%.

chromosomes (Paralogs 1, 3). This case is consistent with previous reports: they likely derived from a common ancestor that first underwent a local duplication to generate two tandem copies constituting a cluster, the copies then underwent a second duplication in genomic range with the couple as a single unit, finally generating the present organization of paralogous clusters after complicated divergent evolution processes [19]. This evolutionary pattern might be explained by their important biological functions: the first duplication could ensure continued activity if one of the copies was unexpectedly destroyed, and the subsequent series of duplications might contribute to their expression specificity in different tissues or at different developmental stages. Interestingly, some residues (at least two in tandem) located at the 3′ ends of related sequences among different clusters in one paralogous group also scored high in the alignment. These conserved sequences remaining during divergent evolution might play important roles in miRNA-mediated developmental and physiological processes.

### *General expression properties of human miRNA clusters in leukemia cell lines*

The expression consistency of miRNAs in a single cluster has been shown in some previous studies [23]. However, a full-scale perspective on the expression of human miRNA clusters is not yet available. In this study, we performed Northern blot analysis of all 148 clustered miRNAs in 6 distinct human leukemia cell lines: 3D5, Jurkat, K562, CMK, HL-60, and U937, classified as B lymphoid, T lymphoid, erythroid, megakaryocytic, myelocytic, and monocytic cell line, respectively, based on original tissue. All results are summarized in a supplemental table (Table S2) and partial blot results are given in Table 3. In addition, specific attention was paid to the 9 paralogous groups of miRNA clusters (Table 2) throughout this analysis. Of all 51 analyzed clusters, 39 showed basically consistent expression patterns in 6 distinct human hematopoietic cell lines (Table S2), such as the mir-17 cluster, the mir-16-1 cluster, and the mir-143 cluster (Fig. 2 and Table 3).

Remarkably, those paralogous clusters, which are located on different chromosomes, displayed a dramatically consistent expression pattern: for example, paralogous group 1 including the let-7a-1, let-7a-3, and mir-98 clusters; paralogous group 2, including the mir-29c and mir-29a clusters; and paralogous group 3, including the mir-15b cluster and mir-16-1 clusters (Table 2). This result is also in line with our previous hypothesis that these paralogous clusters, generated during complex miRNAs evolution, may play more important roles than non-paralogous ones.

To further confirm the expression tendency of these human cluster paralogs in diverse human hematopoietic cell lines, we analyzed four additional human hematopoietic cell lines, HUT-78, Raji, THP-1, and HEL, and one non-hematopoietic cell line, HeLa. In the group of let-7a-1, let-7a-3, and mir-98 clusters, which are respectively located at chromosomes 9, 22, and X, all 6 miRNAs were highly expressed in THP-1 and HeLa, moderately

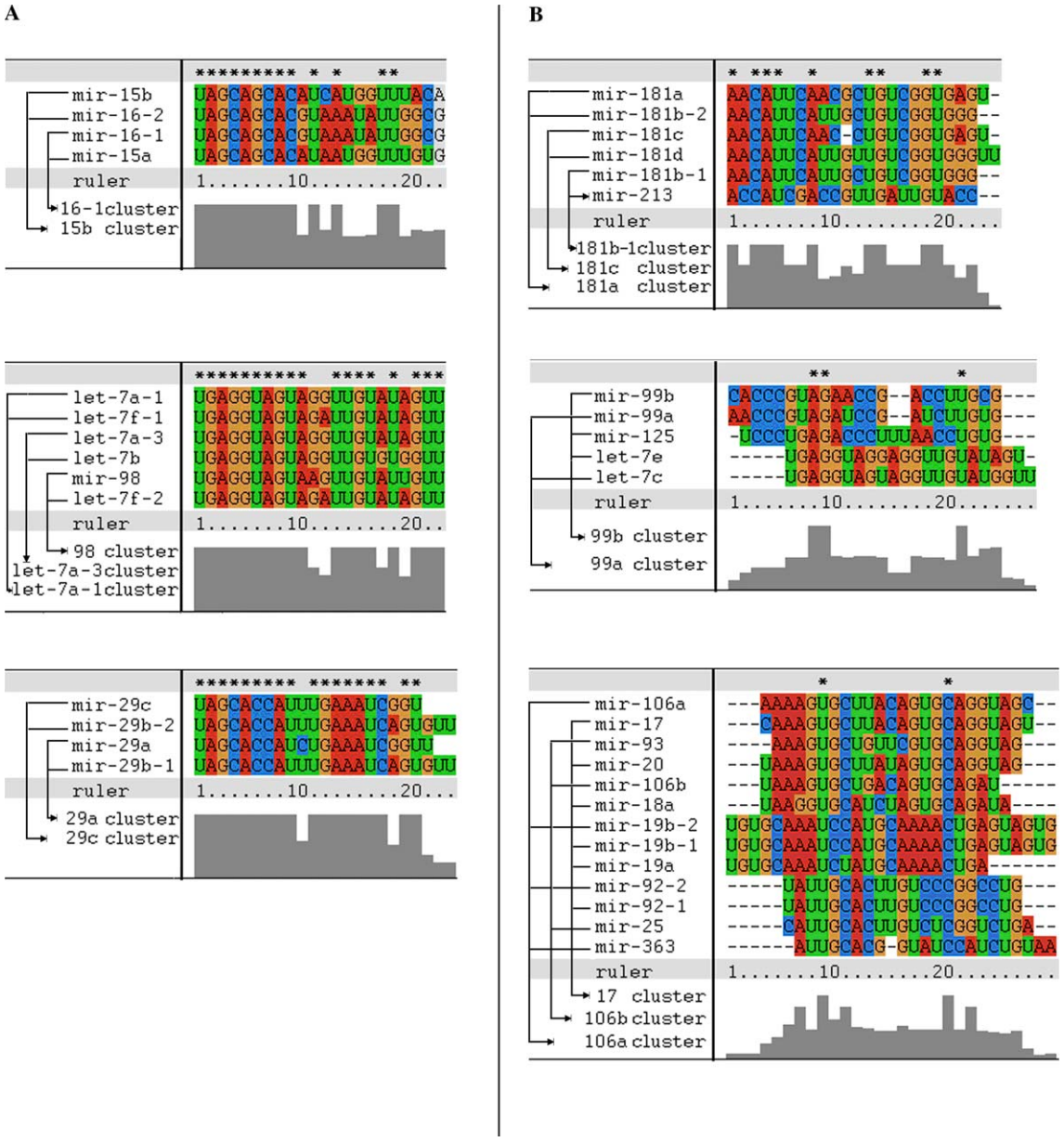


Fig. 1. Sequence alignment of homologous miRNAs in paralogous clusters. Conservation of each nucleotide position is denoted by the color according to ClustalW software. (A) Three groups containing highly homologous (>90%) sequences of clustered miRNAs. (B) Three groups containing partially homologous (50–90%) sequences of clustered miRNAs that constitute distinct paralogs.

expressed in Jurkat and Raji, expressed at lower levels in 3D5, CMK, HEL, HL-60, and U937, and barely detectable in HUT-78 and K562 (Fig. 3B). The mir-29 family in paralog 2 also showed nearly perfect expression coherence with high enrichment in the Jurkat and CMK cell lines (Fig. 3B). But one special exception among paralogous clusters is the group of mir-181b-1, mir-181a, and mir-181c clusters, whose members did not demonstrate completely parallel expression in the diverse hematopoietic cell lines. Mir-181 has been reported to be restricted to murine B cells and to stimulate B-lymphoid cell commitment [10]. However, among the human homologs of mouse, mir-181, mir-181a, and mir-181c were richly expressed in the two T cell lines (Jurkat and Hut-78), while mir-181d was detected in the Jurkat, Hut-78, THP-1, and HeLa cell lines; however, no expression of mir-181b-1, mir-213, and mir-181b-2 was detected in all cell lines tested (Fig. 3B). Species variation may imply differential roles played by homologous miRNAs in human and murine hematopoietic cells.

Moreover, we elucidated another remarkable phenomenon: there was variant signal intensity in the added cell lines belonging to same hematopoietic lineage but from different developmental stages or tissue origins, such as the B cell leukemia cell lines 3D5 and Raji, the T cell leukemia cell lines Jurkat and HUT-78, the erythroleukemia cell lines K562 and HEL, and the monocytic leukemia cell lines U937 and THP-1 (Fig. 3B). For example, differential expression level of the let-7a-1, let-7a-3, and mir-98 clusters

Table 3
Partial expression data of miRNA clusters in six leukemia cell lines

| Cluster | Mapping | miRNA | 3D5 | Jurkat | K562 | CMK | HL-60 | U937 |
|---|---|---|---|---|---|---|---|---|
| 200b-cluster | 1p36.33 | hsa-mir-200b | + | ++++ | + | ++ | – | ++++ |
| | | hsa-mir-200a | + | ++++ | + | ++ | – | ++++ |
| | | hsa-mir-429 | ++ | ++++ | ++++ | ± | ± | ++++ |
| 181b-1-cluster | 1q31.3 | hsa-mir-181b-1 | – | – | – | – | – | – |
| | | hsa-mir-213 | – | + | – | – | – | – |
| 29c-cluster | 1q32.2 | hsa-mir-29c | ± | ++ | – | + | – | + |
| | | hsa-mir-29b-2 | – | ++ | – | ++ | – | + |
| 15b-cluster | 3q25.33 | hsa-mir-15b | +++ | +++ | + | +++ | +++ | +++ |
| | | hsa-mir-16-2 | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ |
| 425-cluster | 3p21.31 | hsa-mir-425 | – | – | – | – | – | – |
| | | hsa-mir-191 | ± | ++++ | ± | ± | ± | ± |
| | | hsa-mir-191* | ± | ++++ | ± | ++++ | ++++ | ++++ |
| 302-cluster | 4q25 | hsa-mir-367 | – | – | – | – | – | – |
| | | hsa-mir-302d | – | ± | – | – | – | ± |
| | | hsa-mir-302a | – | + | – | – | – | ± |
| | | hsa-mir-302a* | ± | + | – | + | ± | ± |
| | | hsa-mir-302c | ± | ++ | – | ++ | ± | ++ |
| | | hsa-mir-302c* | ± | ++ | – | ++ | ± | ++ |
| | | hsa-mir-302b | – | – | – | – | – | – |
| | | hsa-mir-302b* | ++ | + | – | ++ | – | ++ |
| 143-cluster | 5q32 | hsa-mir-143 | ± | ++ | +++ | + | + | +++ |
| | | hsa-mir-145 | ± | ++ | +++ | + | + | +++ |
| 29a-cluster | 7q32.3 | hsa-mir-29a | – | +++ | – | ++ | – | ± |
| | | hsa-mir-29b-1 | – | ++ | – | ++ | – | – |
| let-7a-1-cluster | 9q22.32 | let-7a-1 | – | ++ | – | – | ± | – |
| | | let-7f-1 | ± | +++ | – | + | + | ± |
| 181a-cluster | 9q33.3 | hsa-mir-181a | – | ++++ | – | – | – | – |
| | | hsa-mir-181b-2 | – | – | – | – | – | – |
| 34b-cluster | 11q23.1 | hsa-mir-34b | ++ | – | – | – | – | – |
| | | hsa-mir-34c | + | – | – | – | – | – |
| 17-cluster | 13q31.3 | hsa-mir-17-3p | – | ± | + | – | – | + |
| | | hsa-mir-17-5p | ++ | ++ | ++++ | + | + | +++ |
| | | hsa-mir-18 | – | ++ | ++++ | + | + | ++++ |
| | | hsa-mir-19a | – | ± | ++ | ± | ± | ++ |
| | | hsa-mir-20 | – | + | ++++ | + | + | ++++ |
| | | hsa-mir-19b-1 | – | + | ++++ | + | + | +++ |
| | | hsa-mir-92-1 | – | + | +++ | + | ± | +++ |
| 16-1-cluster | 13q14.2 | hsa-mir-16-1 | +++ | +++ | +++ | +++ | ++ | +++ |
| | | hsa-mir-15a | +++ | +++ | +++ | +++ | ++ | +++ |
| 127-cluster | 14q32.31 | hsa-mir-127 | – | – | – | – | – | – |
| | | hsa-mir-136 | ± | ++++ | ± | ± | ± | ± |
| 23a-cluster | 19p13.12 | hsa-mir-23a | + | – | + | – | + | +++ |
| | | hsa-mir-27a | + | – | + | – | + | +++ |
| | | hsa-mir-24-2 | – | – | – | – | – | – |
| 99b-cluster | 19q13.41 | hsa-mir-99b | – | – | – | – | – | – |
| | | hsa-mir-125a | – | – | – | – | – | – |
| | | let-7e | + | ++++ | – | + | ± | + |
| 181c-cluster | 19p13.12 | hsa-mir-181c | – | ++++ | – | – | – | – |
| | | hsa-mir-181d | – | ++ | ++ | – | – | +++ |
| 371-cluster | 19q13.42 | hsa-mir-371 | – | – | – | – | – | – |
| | | hsa-mir-372 | – | – | – | – | – | – |
| | | hsa-mir-373 | + | – | + | – | +++ | + |
| | | hsa-mir-373* | – | – | – | – | – | – |
| let-7a-3-cluster | 22q13.31 | let-7a-3 | – | + | ± | – | ± | – |
| | | let-7b | ± | ++ | – | – | ± | – |
| 106a-cluster | Xq26.2 | hsa-mir-363 | – | +++ | – | – | ± | – |
| | | hsa-mir-92-2 | ± | + | +++ | ± | ± | +++ |
| | | hsa-mir-19b-2 | ± | + | +++ | + | + | +++ |
| | | hsa-mir-106a | – | – | +++ | – | – | ++ |
| 98-cluster | Xp11.22 | hsa-mir-98 | + | +++ | – | + | + | + |
| | | let-7f-2 | + | +++ | – | + | ++ | + |

Northern blot hybridization signals were normalized and then scored using an arbitrary scale from undetectable (–) to strong (++++) to indicate relative signal intensity in each cell lines.

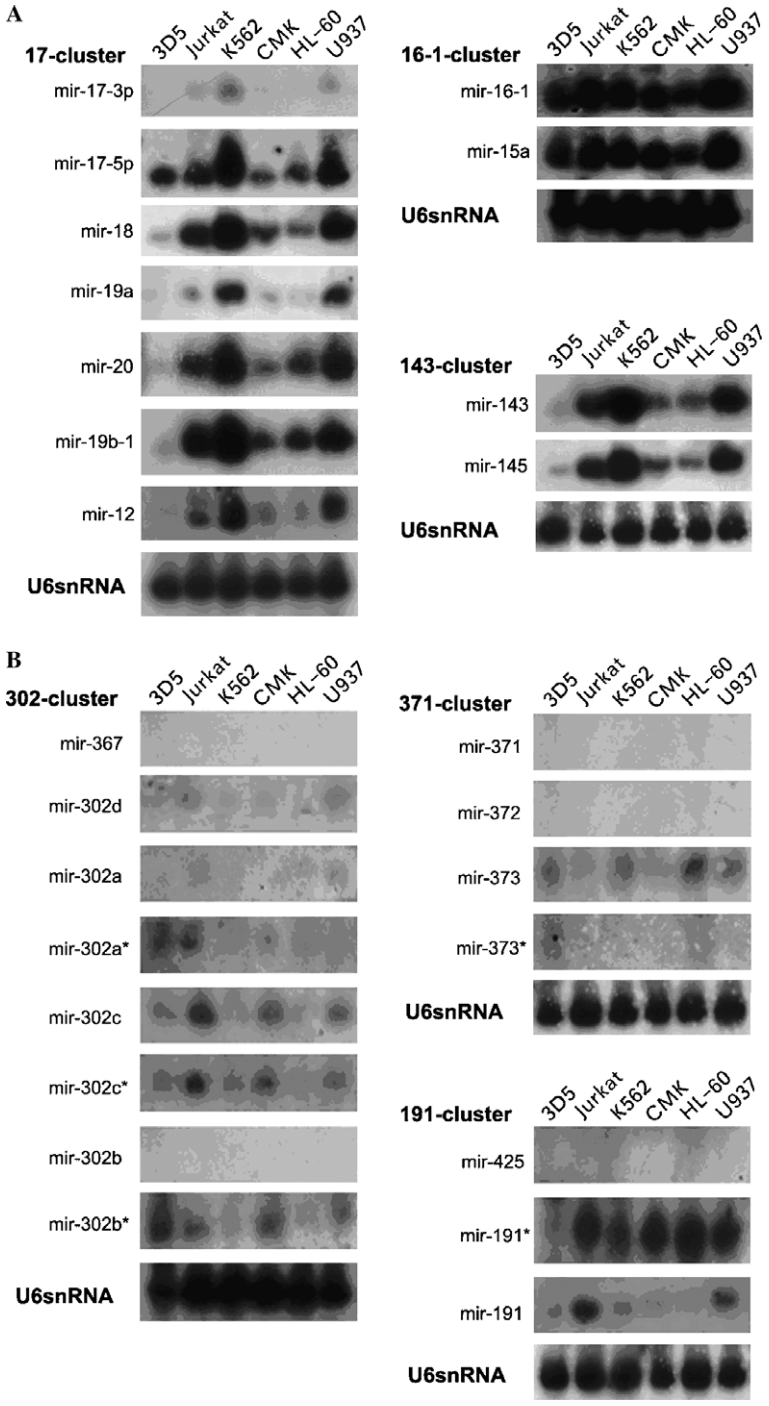


Fig. 2. Expression of miRNA clusters showing nearly consistent (A) and inconsistent (B) tendency. Northern blot analysis of each member in the same miRNA cluster in various hematopoietic cell lines: 3D5, Jurkat, K562, CMK, HL-60, and U937. After the hybridization result was obtained for each miRNA probe, the blot was boiled in 10 mM Tris/mM EDTA, pH7.5, for 5 min and then re-probed for U6 snRNA as an indicator of equal RNA loading (only one is shown here).

was detected in K562 and HEL cell lines, which both represented the erythroid lineage, but K562 was arrested at an earlier stage of erythroid differentiation (Fig. 3B). These expression variations could be explained by the temporal-specific miRNA expression during maturation of the committed cell lineages, implying the possibility that they participated in human erythroid formation.

Nevertheless, inconsistent expression of the mi-RNA members in a single cluster was shown in 11 clusters (Table S2); these included clusters such as the mir-302, mir-371, and mir-191 clusters (Fig. 2B). This phenomenon may be caused by their independent transcription or because they are transcribed together but processed by additional post-transcriptional regulation mechanisms.

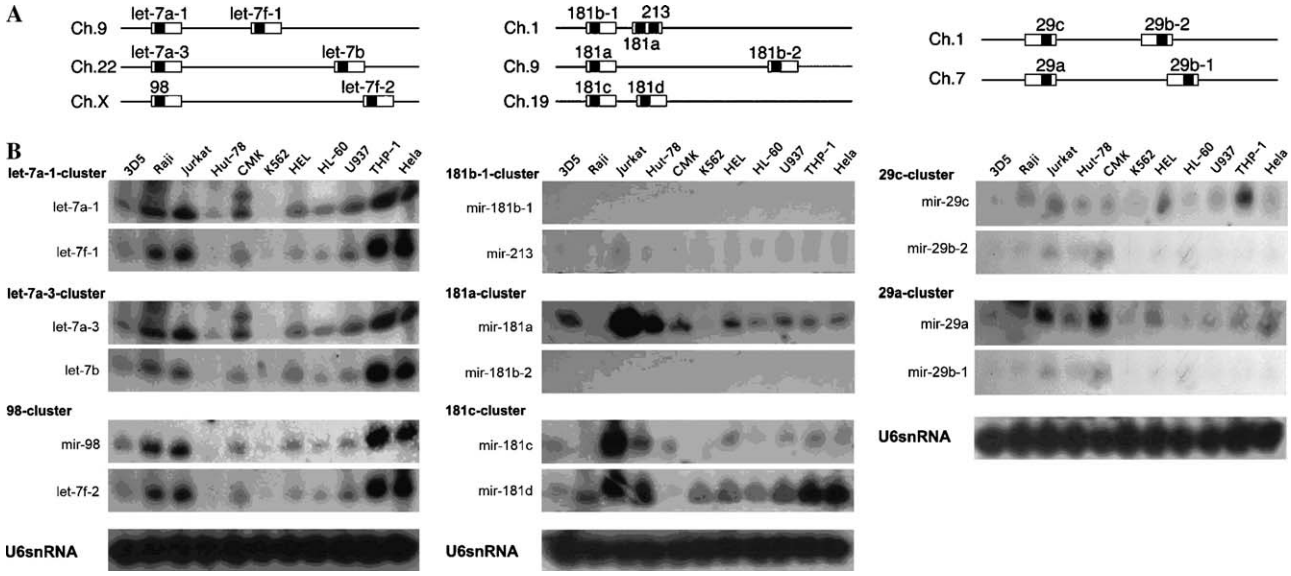


Fig. 3. Expression patterns of miRNA clusters that form paralogs in various hematopoietic cell lines. (A) The genomic organization of miRNA clusters (let-7a-1/let-7a-3/mir-98, mir-181b-1/181a/181c, and mir-181b-1/181a/181c clusters) in three paralogous miRNA cluster groups. The regions corresponding to the miRNA precursors are indicated by boxes and the regions corresponding to mature miRNAs are indicated by black boxes. (B) Northern blot data for the individual miRNA in diverse cell lines are shown. The blots were also re-probed for U6 snRNA, for an indicator of equal RNA loading.

Another noticeable aspect is that many pairs of miRNAs (miRNA:miRNA*) that, respectively, derived from the 5′ and 3′ arms of a common precursor display diverse expression profiles. For example, in the mir-302 cluster, mir-302c, and −302c* are equally expressed, but expression of mir-302a* and mir-302b* can be detected while their partners (mir-302a and mir-302b) have no or low signals. In the mir-371 cluster, mir-373 was expressed in most cell lines while mir-373* was invisible in all the cell lines tested. In the mir-191 cluster, mir-191, and mir-191* also displayed differential expression patterns (Fig. 2B). According to generally accepted ideas, with two mature miRNAs asymmetrically derived from the 5′ and 3′ arms of their common stem-loop precursor, that strand (miRNA) of the duplex whose 5′ end is less tightly paired than the 3′ end (miRNA*) is inclined to enter the RISC/miRNP complexes. The predominance of miRNA* expression of the mir-302 family in the hematopoietic cell lines may indicate the existence of additional factors involved in the choice of the two strands to be assembled into the RISC/miRNP complexes.

Since paralogous miRNAs are very close in sequence, it is important to ensure that an oligo-probing for one sequence could not be affected by another one which has similar sequence. There is only one nucleotide difference between mir-23a and 23b belonging to the mir-23 family, as well as between mir-27a and 27b belonging to mir-27 family. As shown in Fig. 4, distinct expression pattern between two members in each family were clearly presented, which indicated the sensitivity of Northern blot hybridization performed in this study.

### *Identification of hematopoietic lineage-specific or -enriched miRNA clusters and individual miRNAs*

Northern blots, in which DNA oligonucleotides respectively complementary to the 148 miRNAs were used as probes, elicited a universal expression profile of human miRNA clusters in 6 diverse hematopoietic cell lines. A total of 29 clusters showed striking signals in different cell lines, while expression of the other 22 clusters was undetectable even after long exposure to film (Table S2). These results also provided a valuable database of the specificity of miRNA expression in human hematopoietic cell lineages. In detail, several line-specific or -enriched miRNA clusters were identified, implying a potential function of these clusters in human hematopoietic lineage specification and maintenance or even in leukaemogenesis. Typically, mir-17 cluster expression was the highest in erythroid (K562) and monocytic (U937) cell lines, moderate in the T lymphoid cell line (Jurkat), and low or undetectable in the other 3 cell lines (Fig. 2A). This cluster, reported to be upregulated with 13q31–q32 amplification in human B cell lymphomas, was enriched in the K562 and U937 cell lines, which are, respectively, of erythroid and monocytic origin, suggesting additional roles for this cluster in the erythroid and monocytic lineages [15,26]. The mir-143 cluster was also strongly expressed in the K562 and U937 cell lines and moderate in Jurkat (Fig. 2A). Expression of three highly homologous clusters (let-7a-1, let-7a-3, and mir-98) was highly enriched in lymphoid (Raji and Jurkat) and monocytic (THP-1) cell lines (Fig. 3B). The mir-29a cluster showed dominant expression in the Jurkat and CMK cell lines (Fig. 3B).

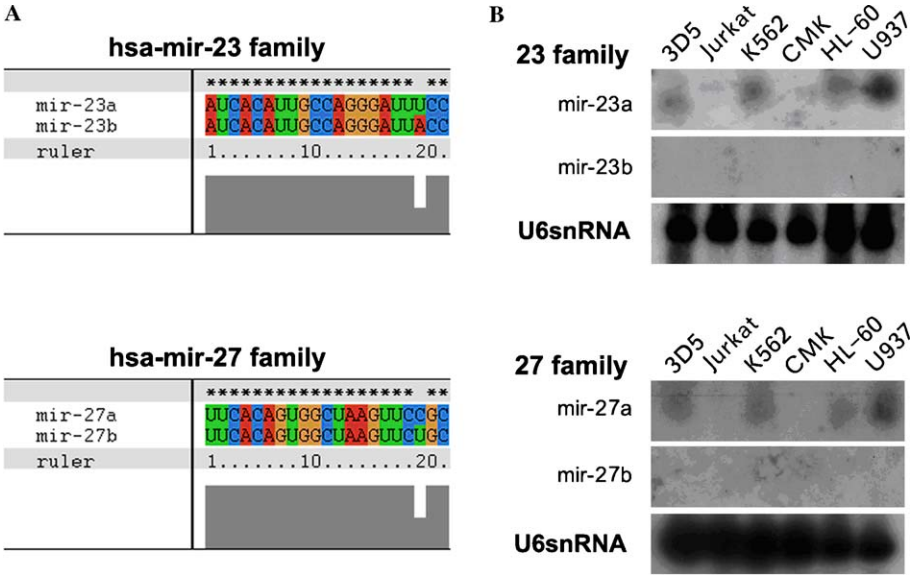


Fig. 4. Verification of hybridization sensitivity. (A) Sequence alignment of has-mir-23 and has-mir-27 families. (B) Northern blots of hsa-mir-23 and has-mir-27 families in 6 leukemia cell lines. The blots were also re-probed for U6 snRNA, for an indicator of equal RNA loading.

By Northern blot analysis, we totally identified 8 hematopoietic cell line-specific or -enriched miRNA clusters; these were, the mir-200b cluster in both the Jurkat and U937 cell lines, the mir-29a cluster in the Jurkat and CMK cell lines, the let-7a-1, let-7a-3, and mir-98 clusters in the Raji, Jurkat, and THP-1 cell lines (Fig. 3B and Table 3), the mir-143 and mir-17 clusters in both the K562 and U937 cell lines (Fig. 2A and Table 3), and the mir-34b cluster in the 3D5 cell line (Table 3).

In addition, we also identified some novel hematopoietic line-specific or -enriched individual miRNAs in inconsistently expressed clusters. For example, mir-191 was richly expressed in the Jurkat and U937 cell lines (Fig. 2B and Table 3), and mir-181c, let-7c, let-7e, and mir-136 were highly expressed in the Jurkat cell line (Fig. 5). Interestingly, more miRNA clusters and individual miRNAs showed expression predominance in Jurkat cell line.

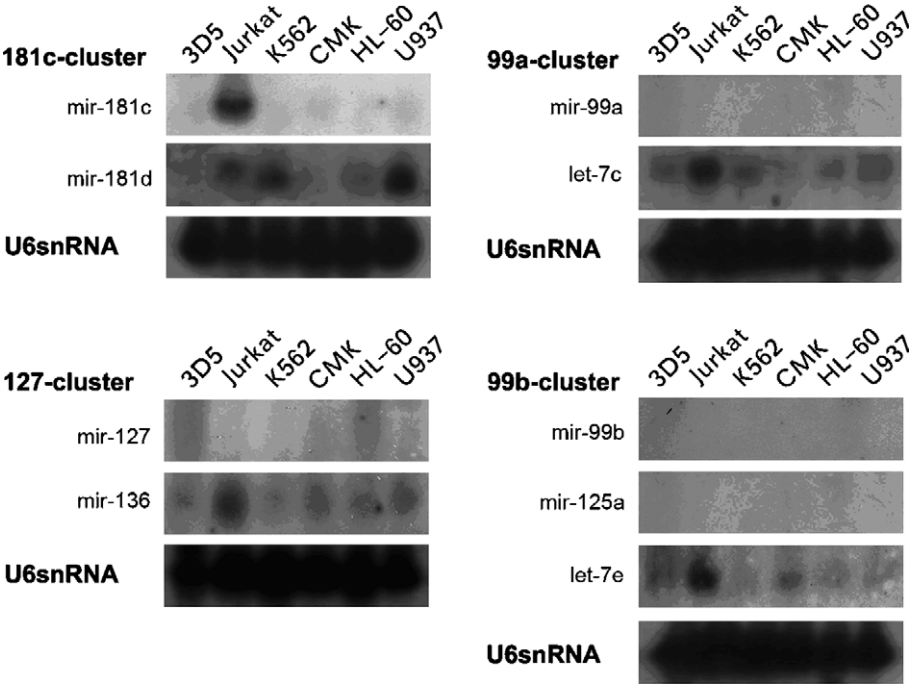


Fig. 5. Northern blots of individual miRNAs that displayed cell line-specific or -enriched expression. U6 snRNA was used as an indicator of equal RNA loading.

Although the number of cell lines used in this study was limited, these results also highlight the potential roles of these miRNAs in maturation of their corresponding lineages and lay a solid foundation for further studies of their regulating actions. All the cells in the hematopoietic system are derived from a common precursor (HSC), which must pass through several critical decision points to give rise to mature blood cells. Two of the most important decision points are the differentiation from common lymphoid progenitors to T cells or B cells, and the differentiation of common myeloid progenitors to granulocyte/monocyte or erythrocyte/megakaryocytes [27]. When the progenitors choose a specific cell fate, access to other lineages must be closed: this closure is achieved by inactivation of some genes that were expressed in these lineages. Those miRNA clusters or individual miRNAs that showed lineage-specific or -enriched expression tendencies may participate in the process of maintaining the lineage specification.

In conclusion, our study provides expression data for human miRNA clusters on a global scale. Although we do not provide unequivocal evidence of the role of miRNAs in normal hematopoiesis, the expression specificity in different cell lines and the reported roles of some miRNAs strongly suggest their involvement in human hematopoiesis.

## Acknowledgments

This work was supported by the National Nature Science Foundation of China (30570389) and the National High Technology Research and Development of China (2002AA223071).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.07.207.

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