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Human microRNA clusters: Genomic organization and expression profile in leukemia cell lines

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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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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