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Dynamic transcriptomes of human myeloid leukemia cells

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ABSTRACT

To identify the mechanisms controlling chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) in humans, we analyzed genome-wide transcription dynamics in three myeloid leukemia cell lines (K562, HL-60, and THP1) using high-throughput sequencing technology. Using KEGG analysis, we found that the ERK/MAPK, JAK-STAT and ErbB pathways promoted proliferation and metabolism in CML. However, in AML, differentiation and apoptosis blocking resulted in the accumulation of blast cells in marrow. In addition, each cell type had unique characteristics. K562 cells are an ideal model for studying erythroid differentiation and globin gene expression. The chemokine signaling pathway and Fc gamma R-mediated phagocytosis were markedly upregulated in HL-60 cells. In THP1 cells, highly expressed genes ensured strong phagocytosis by monocytes. Further, we provide a new insight into myeloid development. The abundant data sets and well-defined analysis methods will provide a resource and strategy for further investigation of myeloid leukemia.

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1. Introduction

Leukemia is a type of cancer that starts in blood-forming tissue and is characterized by an abnormal increase in white blood cells. The disease is divided into several subgroups according to the type of affected blood cell and its chronic or acute forms. Chronic myeloid leukemia (CML) is characterized by active proliferation of stem cells and peripheral blood granulocytes, presence of immature granulocytes at various development stages, and leukocytosis, among others [1]. The cytogenetic hallmark of CML is the Philadelphia chromosome (Ph) created by a reciprocal translocation between chromosomes 9 and 22 [t(9;22)(q34;q11)] [2]. It leads to the formation of a BCR–ABL fusion protein that exerts a constitutive tyrosine kinase activity crucial for the transforming capacity and development of CML [3]. Acute myeloid leukemia (AML) is characterized by myeloid progenitor cell differentiation disorders, accumulation of different types of blast cells in bone marrow, neutropenia, and thrombocytopenia, among others [4]. Many studies have addressed the classification, diagnosis, pathogenesis, and therapy of leukemia. However, the pathogenesis of leukemia is extraordinarily complicated, and the disease exhibits much variation in cytogenetics, genetics, and response to therapy.

To generate an unprecedented global analysis of AML and CML, we used Applied Biosystem's (ABI) SOLiD sequencing system—one of the mainstream next-generation sequencing technologies—to characterize genome-wide transcription dynamics in three myeloid leukemia cell lines. K562, HL-60 and THP1 are used as cell models for researching CML and AML. K562 cells were derived from a patient with CML in the acute phase [5]. HL-60 and THP1 cells were derived from patients with acute promyelocytic leukemia and acute monocytic leukemia, respectively [5,6]. Among samples from leukemia patients, K562, HL-60, and THP1 cells offer the advantage of clear genetic background, abundant epigenetic data related to histone modification and chromatin conformation [7,8], and ready identification of biological function. We validated the CML and AML pathways in different cells with the aim of providing new insights for investigation of CML and AML pathogenesis.

K562 cells are believed to represent stem cell precursors of the myeloid lineage [9] and can differentiate into early precursors of erythrocytes, granulocytes, and monocytes spontaneously or by exposure to various inducers of differentiation. They are used as a model of hematopoietic differentiation [10,11]. HL-60 cells are at the promyelocyte stage of maturation [5] and can be induced to differentiate in vitro into monocytes [12,13], and THP1 cells contain distinct monocytic markers [6]. Our interests focus not only on the

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different pathways between CML and AML but also on the unique functional characteristics of myeloid cells and distinct gene expression patterns during myeloid development.

Comprehensive, genome-wide expression profiling of these cells is expected to provide a valuable resource for the study of gene regulation in AML and CML. The analysis should be useful for a large segment of the medical research community who use human or other vertebrate models for clinical screening, diagnosis, treatment, and prognostic evaluation of AML and CML or myeloid development.

2. Results

2.1. Gene expression profiles of myeloid cell lines

To systematically assess the transcriptome alterations in myeloid leukemia and myeloid cell function in a genome-wide manner, we used the ribo-minus RNA-sequencing method to acquire transcriptional profiles of K562, HL-60, and THP1 cells. After sequencing, a total of 69,176,277; 48,704,448; and 41,421,928 tags were generated from K562, HL-60, and THP1 cells, respectively. After removing remnant rRNA, we mapped these tags (50 bp in length) to the human genome and exon–exon junction database using ABI's Corona Lite software. In the three libraries, 27,387,550; 16,299,179; and 18,353,927 tags were uniquely mapped to the references (Table 1).

We used uniquely mapped reads for further analysis. The total number of expressed genes (with >5 reads) was 18,476. Among the expressed genes, 12,768; 11,459; and 11,067 mRNAs were detected in K562, HL-60, and THP1 cells, respectively. The coverage for each run was normalized to RPKM values to facilitate quantitative comparison between genes and runs [14]. More than 70% of the genes were medium-abundance ($1 \leq \text{RPKM} < 0$), whereas low-abundance ($0 < \text{RPKM} < 1$) genes constituted <30% of the total genes; high-abundance ($\text{RPKM} \geq 50$) genes were the least frequent (Table 2).

To test the reproducibility of the sequencing data, we quantitated the expression of erythroid-related genes in these three cell populations using quantitative real-time PCR (qRT-PCR). Based on transcript levels for *LYZ*, *MDM2*, *E2F1*, *ERBB2IP*, *PIK3CD*, *KLF1*, *IL8* and *ERBB2* genes, the logarithmic transformations of RPKM values and of qRT-PCR relative copy numbers were compared [15]. Our result showed that RNA-seq data were well-correlated with qRT-PCR data (p-value < 0.0001; $R^2 = 0.737$) (Fig. 1).

2.2. Different pathways between CML and AML

We analyzed metabolic pathways of leukemia using KEGG to distinguish pathways between CML and AML. In CML, the ERK/MAPK and JAK-STAT signaling pathways were more highly activated in K562 than in HL-60 cells (Fig. 2). In cell cycle signaling pathways, MDM2, cyclin D1, CDK46, and E2F were upregulated and expression of their corresponding inhibitory molecules was downregulated or even absent. Further, we examined the activation of the ErbB signaling pathway. We found significant upregulation of ErbB receptors, particularly the ErbB2 receptor in K562 cells, which could activate the ErbB pathway (Fig. S1).

Table 2
Classification of genes by expression abundance.

Library (RPKM)	K562 (%)	HL-60 (%)	THP1 (%)
High (>50)	289 (2.26)	316 (2.76)	169 (1.52)
Medium (1–50)	8988 (70.39)	8794 (76.74)	8125 (73.42)
Low (0–1)	3491 (27.34)	2349 (20.50)	2773 (25.06)

In AML, two pathways were notably upregulated in HL-60 cells compared with K562 cells (Fig. 3). One of these pathways was the PI3K/PKB pathway, which is induced by oncogene c-KIT or Flt3. Although c-KIT expression was unchanged in K562 and HL-60 cells, expression of PI3K, PKM/Alt, IKK, and NF-κB, which are present downstream of c-KIT, was upregulated in HL-60 cells, potentially enhancing the transcription of antiapoptosis genes. Flow cytometry showed that HL-60 can resist apoptosis compared with K562 by the fact that a smaller number of HL-60 cells treated with H_2O_2 (2.9%) was detected than K562 cells treated with H_2O_2 (6.8%) at apoptosis stage (Fig. S2). The other pathway was responsible for the differential blocking of granulocytes due to a gene fusion involving retinoic acid receptor alpha (RARA) and PML or PLZF [16]. Compared with K562 cells, PML and RARA were upregulated in HL-60 cells, potentially inhibiting granulocyte differentiation. Similar to HL-60 cells, PI3K/PKB and cell differentiation blocking pathways were more activated in THP1 than in K562 cells (Fig. S3). Moreover, gene expression changes in the AML pathway from the published microarray datasets of AML and CML patients were similar to those analyzed using RNA-seq datasets in HL-60 and K562 cell lines (Fig. S4), which further supported that PI3K/PKB and cell differentiation blocking pathways play an important role in AML.

2.3. Unique functional characteristics of myeloid cells

Differentially expressed genes are essential for determining unique functions in different cells. RPKM values of 3598, 2015, and 438 genes were higher in K562, HL-60, and THP-1 cells, respectively, than those in other cells. We analyzed the functions of these genes on the basis of GO and KEGG functional classification (Table S1). Upregulated genes in K562 cells were mostly involved in metabolic processes, biological synthesis, catalytic processes, and other basic cellular processes. The expression of early erythroid-related factors (HBE, HBZ, HBG1, HBG2, HBQ, GYPA, and EpoR) and transcription factors (GATA1, GATA2, ZFPM1, NF-E2, TAL-1, and KLF1) was clearly upregulated in K562 cells (Fig. S5). Moreover, KLF family members (KLF1, KLF3, KLF6, KLF8, KLF9, KLF10, KLF11, and KLF12) were upregulated in K562 and had a similar expression pattern in the three cell lines (Fig. S5).

Upregulated genes in HL-60 cells were involved in cell cycle, cell division, chemokine signaling pathway, among others (Table S1). We analyzed the chemokine signaling pathway and Fc gamma R-mediated

Table 1
Tag mapping summary.

Type	K562 Reads	K562 Percentage	HL-60 Reads	HL-60 Percentage	THP1 Reads	THP1 Percentage
Total reads	69,176,277	100%	48,704,448	100%	41,421,928	100%
Filter ribosomal	67,967,967	98.25%	41,018,886	84.22%	36,860,810	88.99%
Total mapping	37,183,840	54.71%	21,910,289	53.42%	23,594,340	64.01%
Multiple	9,796,290	26.35%	5,611,110	25.61%	5,240,413	22.21%
Unique	27,387,550	73.65%	16,299,179	74.39%	18,353,927	77.79%
Exon	14,120,740	51.56%	8,561,613	52.53%	9,488,637	51.70%
Intron	10,492,342	38.31%	5,586,017	34.27%	8,051,238	43.87%
Junction	1,239,487	4.53%	797,772	4.89%	609,477	3.32%
Gene-reverse	1,815,027	6.63%	2,260,020	13.87%	2,854,973	15.56%

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