Screening of prognostic risk microRNAs for acute myeloid leukemia

Hai-Yan Gaoa*, Wei Wanga*, Xin-Guo Luob, Yong-Fang Jiangb, Xin Hea, Ping Xu, Xi Chen and Xiao-Yun Lia

aDepartment of Hematology, The Second Affiliated Hospital of Harbin Medical University, Harbin, People’s Republic of China; bDepartment of Hematology, Jinhua People’s Hospital, Jinhua, People’s Republic of China

ABSTRACT

Objectives: This study aimed to investigate the risk miRNAs (microRNAs) for AML (acute myeloid leukemia) prognosis and related regulatory mechanisms.

Methods: MiRNA and gene expression data, as well as clinical data of 176 patients were first downloaded from TCGA. Then miRNAs and genes significantly affecting the survival time based on KM survival curve were identified using Log Rank test. Next, COX proportional-hazard regression analysis was performed to screen the risk miRNAs (P-value < 0.05). Common genes from survival analysis and predicted by miRWalk were used to construct the miRNA regulatory network with the risk miRNAs. Finally, a protein-protein interaction (PPI) network was constructed, as well as functional annotation and pathway enrichment analysis.

Results: The survival analysis revealed 33 miRNAs and 1,377 genes significantly affecting the survival time. HR values of nine miRNAs (up-regulated hsa-mir-606, 520a, 137, 362, 599, 600, 202, 639 and down-regulated 502) were either >1 or <1. The miRNA regulatory network contained 477 nodes and 944 edges. The top ten genes of the constructed PPI network were EGFR, EIF4G1, REL, TOP1, COL14A1, HDAC3, MRPL49, PSMA2, TOP2A and VCAM1 successively. According to pathway enrichment analysis, 6 KEGG pathways and 6 REACTOME pathways were obtained respectively.

Conclusion: Up-regulated hsa-mir-520a, 599, 606, 137 and 362 may increase the prognostic risk for AML patients via regulating the expression of corresponding target genes, especially COL14A1, HDAC3, REL, EGFR, PSMA2, EIF4G1, MRPL49 and TOP1.

KEYWORDS
Acute myeloid leukemia; prognostic risk; MicroRNA; expression regulation

Introduction

Myeloid leukemias, including acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), are clonal bone marrow diseases characterized by the pathological proliferation of abnormal white blood cells [1]. Many studies have investigated the chromosomal abnormalities, gene mutations and epigenetic alterations (DNA methylation and histone modifications) in leukemogenesis [2]. Aberrant methylation of the cytosines at the palindromic CpG sites clustered in gene promoter regions is frequently observed in AML [3,4], and the DNA methylation patterns have been further demonstrated to correlate with prognosis of AML patients [5,6].

miRNA (microRNAs) are small non-coding RNAs 19–25 nucleotides in length, which regulate gene expression generally by inhibiting the translation or inducing the degradation of mRNA, usually via binding to the 3′-untranslated regions (UTRs) of target miRNAs [7]. However, miRNAs can also up-regulate gene expression post-transcriptionally [8]. MiRNA expression profiling has identified miRNA signatures associated with diagnosis, staging and prognosis in many different types of cancer [9]. In leukemia, expression profiles of just 4 miRNAs can reliably distinguish between AML and acute lymphoblastic leukemia (ALL) [10]. In AML, miRNA signatures have been successfully used to distinguish AML cytogenetic subtypes and even molecular subtypes [11,12]. Furthermore, several miRNAs have been identified to be able to predict the survival of AML patients [13,14].

However, prognostic risk factors of AML have never been reported in combination with their regulatory mechanisms. In the present study, based on microRNA sequencing data and gene microarray data, we attempted to investigate risk miRNAs for AML prognosis, as well as their possible regulatory mechanisms, via statistical methods, in order to further understand the prognostic molecular mechanisms.

Methods

Data source

There were microRNA expression data of 188 AML patients (high-throughput sequencing platform: BCGSC_IlluminaGA_miRNASeq), gene expression data of 197 AML patients (annotation platform: Affymetrix HG-U133 Plus 2.0) and clinical data of 200 AML patients in the TGGA database (http://cancergenome.nih.gov/) till 6 August 2014.
Finally, miRNA expression data, gene expression data and clinical data from the same 176 patients were downloaded and used in the present study.

Microarray data pre-processing

The gene expression data were first subject to log2 transformation, followed by the quantile normalization using preprocessCore package of R [15].

Survival analysis

For each miRNA, its average expression value among the 176 AML samples was calculated, which was then taken as the threshold to divide the samples into two groups, namely patients with miRNA expression values higher than the threshold, and those with values lower than the threshold.

Then, KM curve was drawn for each miRNA using KMsurv package of R, and the significant difference between the two groups was determined using Log Rank test. MiRNAs exhibiting significant differences were preliminarily selected as candidate factors affecting prognosis.

Similarly, a survival analysis of gene expression data was also performed. First, the average expression value of each gene of among the 176 samples was calculated, which was also used as the threshold to classify the patients into two groups: patients with gene expression higher than the threshold, and those with values lower than the threshold. Then, KM curve was drawn for each gene using KMsurv package of R, and the significant difference between the two groups was determined using Log Rank test. Genes revealing significant difference were preliminarily selected as candidate factors affecting prognosis.

Evaluation of risk factors using COX model

A COX proportional-hazards regression analysis of the preliminarily screened miRNAs was performed using the stepwise regression method supported by the Survival package of R [16]. MicroRNAs with HR (hazard ratio) values < 1 and > 1 were considered to be the protective and risk factors for AML prognosis respectively.

Construction of miRNA regulatory network

The target genes of miRNA significantly affecting AML prognosis were predicted using miRWalk database [17], which provides ten different prediction algorithms (DIANAmT, miRand, miRDB, miRWalk, RNAhybrid, PICTAR4, PICTAR5, PITA, RNA22 and Targetscan). The target genes predicted by at least five algorithms were selected and further compared with genes that were supposed to affect the survival time according to survival analysis, and the common genes were used to construct the miRNA regulatory network. The resulting network was visualized using cytoscape [18].

Construction of protein-protein interaction (PPI) network of the predicted miRNA target genes

To better understand the interactive relationship between the predicted miRNA target genes, we used STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database to build a PPI network of the predicted miRNA target genes [19]. Genes with combined score of 0.4 or larger was selected to construct the network, which was visualized using Cytoscape software [18]. Additionally, the connection degree of each protein in the network was calculated.

Functional annotation and pathway enrichment analysis of genes included in the PPI network

Genes included in the PPI network were subject to the functional annotation analysis based on GO (gene ontology) database \( P < 0.05 \), as well as the pathway enrichment analysis based on KEGG database (Kyoto Encyclopedia of Genes and Genomes) and REACTOME database using DAVID 7 (Database for Annotation, Visualization and Integrated Discovery, version 7) [20]. Furthermore, a pathway-pathway connection network was also constructed based on commonly-enriched genes.

Results

Result of data preprocessing

Totally, the expression data of 705 miRNAs were obtained from the high-throughput sequencing data, and the expression data of 19,800 genes were detected after annotation.

Result of survival analysis

According to the survival analysis, 33 miRNAs that were estimated to significantly affect the survival time were preliminarily screened. For hsa-mir-380, hsa-mir-600, hsa-mir-27b, hsa-mir-128-1, hsa-mir-25, hsa-mir-202, hsa-mir-143, hsa-mir-181b-1, hsa-mir-181b-2, hsa-mir-551a, hsa-mir-639, hsa-mir-191, hsa-let-7d, hsa-mir-181a-1 and hsa-mir-181a-2, when they were highly-expressed, the survival time of AML patients was longer than that when they were lowly-expressed. By contrast, for hsa-mir-17, hsa-mir-606, hsa-mir-532, hsa-mir-767, hsa-mir-19b-2, hsa-mir-520a, hsa-mir-20a, hsa-mir-20b, hsa-mir-502, hsa-mir-506, hsa-mir-137, hsa-mir-513b, hsa-mir-362, hsa-mir-510, hsa-mir-188, hsa-mir-599, hsa-mir-155 and hsa-mir-19a, when they were expressed at higher levels, the survival time of AML patients was significantly shorter, compared to that when they were expressed at lower levels.
Meanwhile, 1,377 genes significantly affecting the survival time were also obtained.

Result of COX proportional-hazards regression

Using the stepwise regression method, 14 out of 33 miRNAs were finally screened and used to perform the COX proportional-hazards regression. The regression result revealed that 12 miRNAs revealed P-value < 0.05 (Table 1). Among them, HR values of patients with up-regulated hsa-mir-606, 520a, 137, 362 and 599 were >1, which were thus considered to be the prognostic risk factors for AML; while HR values of up-regulated has-mir-600, 202, and 639 and down-regulated has-mir-502 were <1, which were thus considered to be prognostic protective factors. In addition, HR values of has-mir-19b-2, 191 and 155 were 1, suggesting that they were neither a risk factor nor a protective factor. The KM curves for the prognostic 9 miRNAs were shown in Figure 1.

Construction of miRNA regulatory network

Using miRWALK, each of the nine miRNAs was predicted to have target genes, and finally 13,836 target genes were screened. Finally, 468 common target genes were obtained after comparing these 13,836 genes with the 1377 genes that were estimated according to survival analysis. Overall, the miRNA regulatory network consisting of 9 miRNA and 468 target gene contained 477 nodes and 944 edges (Figure 2). hsa-mir-520a were estimated to have 240 target genes including COL14A1, HDAC3, REL and EGFR, hsa-mir-599 with 48 genes such as MRPL49, hsa-mir-606 with 76 target genes, such as COL14A1 and TOP2A, has-mir-137 with 100 target genes such as TOP1, and hsa-mir-362 with 156 target genes, including HDAC3, MRPL49, PSMA2 and EIF4G1.

Construction of PPI network of the predicted miRNA target genes

The constructed PPI network of the predicted miRNA target genes contained 406 edges and 256 nodes (Figure 3). The top ten genes with the largest degree of connection to other genes were EGFR (21), EIF4G1 (12), REL (12), TOP1 (12), COL14A1 (11), HDAC3 (11), MRPL49 (11), PSMA2 (11), TOP2A (11) and VCAM1 (11) successively.

Functional annotation and pathway enrichment analysis of genes included in the PPI network

According to pathway enrichment analysis, the genes included in the PPI network were speculated to function via one or more of the following 6 KEGG pathways and 6 REACTOME pathways: endocytosis (hsa04144), insulin signalling pathway (hsa04910), regulation of actin cytoskeleton (hsa04810), ErbB signalling pathway (hsa04012), pathways in cancer (hsa05200), Jak-STAT signalling pathway (hsa04630), signalling by insulin receptor(REACT_498), apoptosis (REACT_578), integrin cell surface interactions (REACT_13552), HIV infection (REACT_6185), signalling by Wnt (REACT_11045) and signalling by PDGF (REACT_16888) (Table 2).

Additionally, almost every two pathways had commonly-enriched genes (Figure 4). For example, EGFR was commonly-enriched in endocytosis, ErbB signalling pathway, regulation of actin cytoskeleton, pathways in cancer; PSMA2 in HIV infection, signalling by Wnt and apoptosis.

Discussion

In the present study, microRNAs hsa-mir-520a, 599, 606, 137 and 362 were speculated to be the risk factors for AML prognosis. Although many miRNAs have been reported to have key roles in AML, hsa-mir-520a, 599 and 606 have been seldom reported in AML. They were observed to increase the AML prognostic risk via regulating many target genes. According to the constructed PPI network, EGFR, EIF4G1, REL, TOP1, COL14A1, HDAC3, MRPL49, PSMA2, TOP2A and VCAM1 may have critical effects on AML prognosis, especially EGFR and PSMA2, which may affect the prognostic condition via multiple pathways. Additionally,

Table 1. Result of COX proportional-hazards regression analysis.

| Factor       | β     | HR    | P-value | Lower 95% | Upper 95% |
|--------------|-------|-------|---------|-----------|-----------|
| hsa-mir-600  | -0.3155 | 0.7308| 0.005291| 0.5863    | 0.911     |
| hsa-mir-602  | 0.001231| 1.964 | 0.000223| 1.4365    | 2.6844    |
| hsa-mir-520a | -0.1942 | 0.8235| 0.000247| 0.7262    | 0.9339    |
| hsa-mir-520a | 0.9302  | 2.535 | 0.032036| 1.0831    | 5.9331    |
| hsa-mir-137  | -0.2096 | 0.9793| 0.024892| 0.9615    | 0.9974    |
| hsa-mir-513b | 0.3158  | 1.171 | 0.0000425| 1.1986    | 1.5688    |
| hsa-mir-513b | 0.2008  | 1.222 | 0.065848| 0.9869    | 1.5139    |
| hsa-mir-362  | 0.0259  | 1.026 | 0.01111 | 1.0104    | 1.0423    |
| hsa-mir-188  | 0.02779 | 1.028 | 0.063624| 0.9984    | 1.0588    |
| hsa-mir-599  | 0.8558  | 2.353 | 0.037267| 1.0518    | 5.2651    |
| hsa-mir-639  | -0.2328 | 0.7923| 0.0332| 0.6783    | 0.9256    |
| hsa-mir-191  | 0.00002745| 1.000444| 1.0001    |
each gene may be regulated by more than one risk miRNA to affect the AML prognosis.

Hsa-mir-520a was predicted to be the most important risk factor for AML prognosis. Previously, its down-regulation has been reported to correlate with the Imatinib resistance in chronic myeloid leukemia patients [21], but its role in AML has never been reported so far. In the present study, this miRNA was speculated to increase the prognostic risk of AML via regulating various genes, especially \textit{EGFR}, \textit{REL}, \textit{COL14A1} and \textit{HDAC3}. Among them, \textit{EGFR} (epidermal growth factor receptor) was enriched into four pathways, namely endocytosis, regulation of actin cytoskeleton, erbB signalling pathway and pathways in cancer. In the latter two pathways, \textit{Akt} is a downstream gene. Scheepers et al. have reported that inhibition of \textit{EGFR} activity can reduce the AML cell survival via inhibition of downstream \textit{Akt} and \textit{Erk} proteins [22]. In addition, small molecule EGFR-targeted therapy in AML patients such as erlotinib may be related to the inhibition of oncogenic signalling via SRC family kinases and mTOR (downstream signal of Akt) [23]. Given that the outcome of inhibition of \textit{EGFR} is similar to a down-regulation of its expression level, it may be inferred that higher \textit{EGFR} expression level might be conducive to the survival of AML cells, further suggesting that hsa-mir-520a might increase the prognostic risk of AML via up-regulating the \textit{EGFR} level. The proto-oncogene \textit{REL} encodes c-REL, a transcription factor that is a member of the Rel/NFkB family [24]. c-REL has been reported to be a transcriptional target of \textit{MIXL1} (Mesoderm Inducer in Xenopus Like 1), a critical regulator of embryonic and adult hematopoiesis, by Raymond et al. who proposed that the up-regulation of \textit{REL} by \textit{MIXL1} promoted the survival or proliferation of AML cells via activating the NF-κB pathway [25]. This suggests that
hsa-mir-520a might also increase the prognostic risk of AML via up-regulating REL expression. COL14A1 encodes the alpha chain of type XIV collagen that is involved in cell adhesion. It was observed to be up-regulated in DAC (decitabine)-treated AML cells and DAC can induce AML remission via reducing the genomic DNA methylation [26]. This was consistent with the speculation that hsa-mir-520a might increase the AML prognostic risk via down-regulating COL14A1.

HDAC3 (histone deacetylase 3) is one of the four members of the human class I HDACs that catalyze the removal of acetyl groups from lysine residues in histones and nonhistone proteins [27]. Wada et al. have experimentally observed HDAC overexpression and histone hypoacetylation in AML cell lines [28]. Lepore et al. have reported HDAC inhibitor Vorinostat reduces the expression level of BARD1 (BRCA1-associated RING domain 1) isoforms that might act as oncogenes via elevating the expression of miR-19a and miR-19b in AML cells [29]. Thus, it is presumably that high HDAC level may be responsible for the overexpression of BARD1 isoforms. It can be further inferred that miR520a may increase the prognostic risk of AML by increasing HDAC expression level.

Hsa-mir-599 was also an important risk factor for AML prognosis, which has also been identified as a risk factor for the prognosis of pediatric acute lymphoblastic leukemia by Schotte et al [30]. MRPL49 (mitochondrial ribosomal protein L49) was predicted to be among its target genes, which has only been reported to be up-regulated in human breast cancer [31]. According to our study, it was supposed to increase the AML prognostic risk via the signalling by insulin receptor under the up-regulation by hsa-mir-599.

Figure 2. The miRNA-gene regulatory network. A red triangle represents miRNA; a blue ellipse represents a target gene.
Has-miR-606 was another risk factor for AML prognosis. However, whether its aberrant expression level is related to AML prognosis has never been reported. 

COL14A1 and TOP2A were among its target genes. TOP2A (DNA topoisomerase IIa) is an essential proliferation-dependent nuclear enzyme [32]. Beck et al. observed significantly decreased TOP2A level in AML samples [33]. Thus, this suggests that has-miR-606 may increase the prognostic risk of AML via down-regulating this gene.

Has-miR-137 was also predicted to be a prognostic risk factor for AML patients. It has been previously reported to repress the expression of EZH2 [34], and inhibition of the latter was reported to induce apoptosis of AML cells [35]. However, EZH2 was not observed here, thus the role of this miRNA in AML is still unknown. Top 1 was among its multiple targets observed in the present study. It encodes eukaryotic topoisomerase I, an enzyme catalyzes the transient breaking and rejoicing of a single strand of DNA,

Figure 3. The constructed protein-protein interaction network of the predicted miRNA target genes. A blue ellipse represents a target gene of miRNA; an edge between two genes indicates there is an interaction, and the width of the edge is determined by the combined score between the two genes.
thus altering the topology of DNA required during transcription [36]. Top 1 has been known as a NUP98 fusion partner in t(11;20)(p15;q11) that has been detected as a recurring abnormality in AML patients [37]. However, its expression change has never been reported, so how has-miR-137 increases the prognostic risk via regulating its expression needs to be further investigated.

Has-mir-362 was also predicted to be a risk factor for AML prognosis, which is consistent with the finding that one of its mature forms mir-362-3p was greatly overexpressed in AML samples by Ramsingh et al. using deep sequencing [38] and Cammarata et al. using microarrays [39]. In the present study, EIF4G1 (eukaryotic translation initiation factor 4 gamma, 1), HDAC3, MRPL49 (mitochondrial ribosomal protein L49) and PSMA2 (proteasome subunit, alpha type, 2) were predicted to be among the target genes of mir-362. Furthermore, PSMA2 was speculated to function via three pathways: apoptosis, HIV infection and signalling by Wnt. However, little is known on their roles in AML prognosis so far.

MicroRNAs hsa-mir-520a, 599, 606, 137 and 362 may increase the prognostic risk for AML patients via regulating the expression of corresponding target genes, especially COL14A1, HDAC3, REL, EGFR, PSMA2, EIF4G1, MRPL49 and TOP1. However, there were some limitations in the present study. For example, the regulation between miRNAs and their target genes needs to be experimentally validated, especially the prognostic expression change of miRNAs and genes that have

Table 2. Result of functional annotation and pathway enrichment analysis of genes included in the PPI network.

| Pathway                           | P-value  | Gene                                                                 | Benjamini |
|----------------------------------|----------|----------------------------------------------------------------------|-----------|
| hsa04144:Endocytosis             | 0.001934 | EGFR, FGFR2, AP2B1, DNMT1, IL2RA, SH3GLB2, VPS37A, VPS24, PRRC1, PAR6GG, KIT, FZR | 0.223     |
| hsa04910:Insulin signalling pathway | 0.008456 | SOCS3, ARAF, SOCS1, HJ2, MKNK2, PRKCI, PDE3B, RAF1, CRK              | 0.424     |
| hsa04810:Regulation of actin cytoskeleton | 0.017252 | EGFR, FGFR2, ITGA5, PTK2, SH11, RAC2, AP2C, ARAF, RAF1, CRK, FZR     | 0.53      |
| hsa05200:Pathways in cancer      | 0.049716 | FGFR2, EGF, EREG, ARAF, RAF1, CRK                                    | 0.729     |
| hsa04630:Jak-STAT signalling pathway | 0.049774 | LIF, STAT6, IL2RA, SOCS2, IL7, CCND2, SOCS1, PIAS2                   | 0.734     |
| REACT_498:Signalling by Insulin receptor | 0.00676  | EGF, DOK1, YWHAB, PDE3B, RAF1                                        | 0.297     |
| REACT_578:Apoptosis              | 0.012601 | PSMA2, PSME1, PTK2, LMNB1, PSMD11, CASP8, LMNA, YWHAB               | 0.281     |
| REACT_13552:Integrin cell surface interactions | 0.020697 | VCAM1, ITGA5, BSG, PTK2, COL2A1, CRK                                 | 0.304     |
| REACT_6185:HIV Infection         | 0.026642 | TAF11, PSMA2, PSME1, AP2B1, ELL, PSMD11, BTRC, POL1A2D, NUP3S        | 0.296     |
| REACT_1104:Signalling by Wnt      | 0.031069 | PSMA2, PSME1, PSMD11, BTRC, COL1                                     | 0.28      |
| REACT_16888:Signalling by PDGF    | 0.036211 | STAT6, YWHAB, RAF1, COL2A1, CRK                                      | 0.274     |

Figure 4. A pathway-pathway connection network. A circle represents a pathway, and its size is directly determined by the number of enriched genes, and its colour by the P-value, a deeper colour indicating a smaller P-value; a blue edge between two pathways indicates a larger width and a deeper colour indicating they have more common enriched genes.
never been reported in AML based on the patient samples. In the next step, which of the 5 risk miRNAs had highest expression among complex/adverse cytogenetics or p53 mutated AML patients would be identify.

Acknowledgements
This work was supported by the [Youth Science Fund of the Natural Science Foundation of China] under Grant (number 81001051); [Postdoctoral Science Foundation of Heilongjiang Province] under Grant (number LBH-Z15129); [the Yong and middle-aged Science Foundation of Harbin Medical University] under Grant (number CX2016-19); and [Health and Family Planning Commission of Heilongjiang Province] under Grant (number 2016-080).

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This work was supported by the [Youth Science Fund of the Natural Science Foundation of China] under Grant (number 81001051); [Postdoctoral Science Foundation of Heilongjiang Province] under Grant (number LBH-Z15129); [the Yong and middle-aged Science Foundation of Harbin Medical University] under Grant (number CX2016-19); and [Health and Family Planning Commission of Heilongjiang Province] under Grant (number 2016-080).

References
[1] Fröhling S, Scholl C, Gilliland DG, et al. Genetics of myeloid malignancies: pathogenetic and clinical implications. J Clin Oncol. 2005;23(26):6285–6295.
[2] Murati A, Brecqueville M, Devillier R, et al. Myeloid malignancies: mutations, models and management. BMC Cancer. 2012;12(1):304.
[3] Toyota M, Kopecky KJ, Toyota M-Q, et al. Methylation profiling in acute myeloid leukemia. Blood. 2001;97(9):2823–2829.
[4] Kroeger H, Jelinek J, Estécio MR, et al. Aberrant CpG island methylation in acute myeloid leukemia is accentuated at relapse. Blood. 2008;112(4):1366–1373.
[5] Bullinger L, Ehrich M, Döhner K, et al. Quantitative DNA methylation predicts survival in adult acute myeloid leukemia. Cancer Cell. 2010;17(1):13–27.
[6] Guo H, Ingolia NT, Weissman JS, et al. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature. 2010;466(7308):835–840.
[7] Vasudevan S. Posttranscriptional upregulation by microRNAs. WIREs RNA. 2012;3(3):311–330.
[8] Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006;6(11):857–866.
[9] Mi S, Lu J, Sun M, et al. MicroRNA expression signatures accurately discriminate acute lymphoblastic leukemia from acute myeloid leukemia. Proc Natl Acad Sci U S A. 2007;104(50):19971–19976.
[10] Jongen-Lavrencic G, Sun SM, Dijkstra MK, et al. MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. Blood. 2008;111(10):5078–5085.
[11] Dixon-McIver A, East P, Mein CA, et al. Distinctive patterns of microRNA expression associated with karyotype in acute myeloid leukaemia. PloS One. 2008;3(5):e2141.
[12] Garzon R, Volinia S, Liu C-G, et al. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood. 2008;111(6):3183–3189.
[13] Marcus G, Radmacher MD, Maharry K, et al. MicroRNA expression in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358(18):1919–1928.
[14] Bostad B. PreprocessCore: a collection of pre-processing functions. R package version. 2013;1(0).
[15] Therneau TM. Modeling survival data: extending the Cox model. New York: Springer; 2000.
[16] Dweh H, Sticht C, Pandey P, et al. Mirwalk – database: prediction of possible miRNA binding sites by ‘walking’ the genes of three genomes. J Biomed Inform. 2011;44(5):839–847.
[17] Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of molecular interaction networks. Genome Res. 2003;13(11):2494–2504.
[18] Franceschini A, Szklarczyk D, Frankild S, et al. String v9.1: protein–protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2012;41(1):D808–D815.
[19] DennisG, Sherman BT, Hosack DA, et al. David: database for annotation, visualization, and integrated discovery. Genome Biol. 2003;4(5):P3.
[20] San José-Enériz E, Román-Gómez J, Jiménez-Velasco A, et al. MicroRNA expression profiling in imatinib-resistant chronic myeloid leukemia patients without clinically significant ABL1-mutations. Mol Cancer. 2009;8(1):69–72.
[21] Scheepers ER, Kampen K, de Bont ES. Inhibition of downstream Akt and Erk signaling pathways. Cancer Res. 2013;73(8 Supplement):2772.
[22] Koehler S, Galluzzi L, Lainey E, et al. Erlotinib antagonizes constitutive activation of SRC family kinases and mTOR in acute myeloid leukemia. Cell Cycle. 2011;10(18):3168–3175.
[23] Gilmore TD, Kalaitzidis D, Liang M-C, et al. The c-Rel transcription factor and B-cell proliferation: a deal with the devil. Oncogene. 2004;23(13):2275–2286.
[24] Raymond A, Liu B, Liang H, et al. A role for BMP-induced homeobox gene MIXL1 in acute myelogenous leukaemia and identification of type I BMP receptor as a potential target for therapy. Oncotarget. 2014;5(24):12675.
[25] Flotho C, Claus R, Batz C, et al. The DNA methyltransferase inhibitors azacitidine, decitabine and zebularine exert differential effects on cancer gene expression in acute myeloid leukemia cells. Leukemia. 2009;23(6):1019–1028.
[26] Marks PA, Rifkind RA, Richon VM, et al. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer. 2001;1(3):194–202.
[27] Wada T, Kikuchi J, Nishimura N, et al. Expression levels of BARD1 isoform expression in cytogenetically normal acute myeloid leukemia. PLoS One. 2008;3(5):e2141.
[28] Lepore D, Bellissimo MT, Cipolletti M, et al. Hdac inhibitors repress BARD1 isoform expression in acute myeloid leukemia cells via activation of miR-19a and/or b. PLoS One. 2013;8(12):e83018.
[30] Schotte D, De Menezes RX, Moqadam FA, et al. MicroRNAs characterize genetic diversity and drug resistance in pediatric acute lymphoblastic leukemia. Haematologica. 2011;96:703–711. haematol. 2010.026138.

[31] Lamb R, Sneddon S, Hulit J, et al. Mitochondria ‘fuel’ breast cancer metabolism: fifteen markers of mitochondrial biogenesis label epithelial cancer cells, but are excluded from adjacent stromal cells. Cell Cycle. 2012;11(23):4390–4401.

[32] Tsai S-C, Valkov N, Yang W-M, et al. Histone deacetylase interacts directly with DNA topoisomerase II. Nat Genet. 2000;26(3):349–353.

[33] Beck J, Niethammer D, Gekeler V. Mdr1, MRP, topoisomerase IIalpha/beta, and cyclin A gene expression in acute and chronic leukemias. Leukemia. 1996;10:S39–S45.

[34] Juan AH, Kumar RM, Marx JG, et al. Mir-214-dependent regulation of the polycomb protein Ezh2 in skeletal muscle and embryonic stem cells. Mol Cell. 2009;36 (1):61–74.

[35] Fiskus W, Wang Y, Sreekumar A, et al. Combined epigenetic therapy with the histone methyltransferase EZH2 inhibitor 3-deazaneplanocin A and the histone deacetylase inhibitor panobinostat against human AML cells. Blood. 2009;114(13):2733–2743.

[36] Gentry AC, Juul S, Veigaard C, et al. The geometry of DNA supercoils modulates the DNA cleavage activity of human topoisomerase I. Nucleic Acids Res. 2010;39:1014–1022. gkq822.

[37] Potenza L, Sinigaglia B, Luppi M, et al. A t(11;20)(p15;q11) may identify a subset of nontherapy-related acute myelocytic leukemia. Cancer Genet Cytogenet. 2004;149(2):164–168.

[38] Ramsingh G, Koboldt DC, Trissal M, et al. Complete characterization of the microRNAome in a patient with acute myeloid leukemia. Blood. 2010;116(24):5316–5326.

[39] Cammarata G, Augugliaro L, Salemi D, et al. Differential expression of specific microRNA and their targets in acute myeloid leukemia. Am J Hematol. 2010;85 (5):331–339.