

ORIGINAL ARTICLE

MicroRNA *miR-181a* correlates with morphological sub-class of acute myeloid leukaemia and the expression of its target genes in global genome-wide analysis

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MicroRNAs (miRNAs) are short single-stranded RNAs that have a potentially important role in gene regulation. Using a quantitative real-time polymerase chain reaction assay specific to the mature miRNA, the expression level of a selected group of haematopoietic tissue-specific miRNAs was measured across a set of 30 primary adult acute myeloid leukaemia (AML) with a normal karyotype. The expression levels of each miRNA were correlated with the genome-wide mRNA expression profiles in the same leukaemias. This revealed that *miR-181a* correlated strongly with the AML morphological sub-type and with the expression of genes previously identified through sequence analysis as potential interaction targets. Three other miRNAs, *miR-10a*, *miR-10b* and *miR-196a-1*, showed a clear correlation with HOX gene expression.

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Introduction

A large number of short endogenous non-coding RNAs have been identified in species as diverse as plants and mammals and have been designated¹ as microRNAs (miRNAs). These molecules are typically 19–25 nucleotides in length, evolutionarily conserved and appear to play an important role in modulating gene expression. This is because of complementarity-mediated binding to target mRNAs resulting in the repression of translation² or, as new evidence suggests, in the cleavage of the target transcript.^{3–8} Although many of the known human miRNA genes are located at fragile sites and genomic regions involved in cancer, including the HOX gene clusters,^{9,10} their role in the oncogenic process is not fully understood. However, several examples of an association between disrupted expression of specific miRNAs and cancer have been shown.^{11–20} Moreover, Lu *et al.*²¹ first observed distinct patterns of miRNA expression across tumour types and miRNA profiles reflecting the developmental lineage and the differentiation state of the tumour.

The development of the quantitative 'looped' real-time polymerase chain reaction (PCR) assay specific for the mature miRNA expression levels²² has allowed us to investigate the correlation between the expression of endogenous miRNAs and global gene expression patterns of the same cancer cell in primary leukaemias. Using computational methods, we demonstrated that particular miRNA levels are correlated with gene expression profiles in primary adult acute myeloid leukaemia (AML) with a normal karyotype (NK).

Materials and methods*Sample collection*

Upon informed consent, 30 AML samples were obtained from peripheral blood (PB) or bone marrow (BM) of patients in St Bartholomew's Hospital. The morphological diagnosis of leukaemia was made as described previously.²³ Only samples with a blast count greater than 60% in the PB were processed. The median age at diagnosis was 57.2 years (range 19–87); 17 were women, and according to the French American British (FAB) classification,²⁴ there were thirteen M1, seven M2, six M4 and four M5. (Full sample details are described in Supplementary Table S1.)

Gene expression profiling

Total RNA was extracted from a total of 10×10^6 to 20×10^6 thawed cells using Trizol (Invitrogen, Carlsbad, CA, USA) purification method. U133A and U133B oligonucleotide arrays (Affymetrix) were used for the expression profile. cRNA target preparation and array hybridization were performed as described previously.²³ Bioconductor was used to calculate the level of expression for each gene (<http://www.bioconductor.org/>). The data were normalized by quantile normalization, as implemented in the Affymetrix package of Bioconductor. (The normalized values of gene expression of the 30 NK-AML samples for both U133A and U133B arrays are reported in Supplementary Tables S2 and S3, respectively.) All the statistical analysis was performed with the statistical language R (<http://www.R-project.org/>). GeneSpring 6.2 (Silicon Genetics) was used for the calculation and generation of the heat map from the hierarchical cluster analysis that was performed using as a metric Pearson's correlation.

Selection of miRNAs

Five miRNAs were selected from the Sanger miRNA Registry at <http://www.sanger.ac.uk/>²⁵ (Table 1): (a) *miR-181a*, known to be involved in haematopoietic differentiation²⁶ and to target HOX genes;^{27–29} (b) *miR-10a*, *miR-10b* and *miR-196a-1* located within the HOX clusters;^{30,31} and (c) *miR-223*, known to be expressed at a high level in cells of the myeloid lineage,^{26,32} used as normalization control.

Quantitative real-time PCR assay for miRNAs

MicroRNAs were quantitated by real-time PCR using TaqMan MicroRNA assay (Applied Biosystems). All the reagents for performing reverse transcription reaction and the real-time PCR assays specific to the mature miRNAs were provided as part of a collaboration agreement with Applied Biosystems (P/N: 4365409). The reverse transcription reaction (RT) and real-time

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Table 1 List of the miRNAs selected for quantitative measurement

Name	GenBank	Chromosome location	miRNA location (Mb)	Mature miRNAs
<i>miR-10a</i>	AF287967	17q21.3	46.95–47.05	UACCCUGUAGAUCGGAUUUGUG
<i>miR-10b</i>	AC009336	2q31	176.85–177	UACCCUGUAGAACCGAAUUUGU
<i>miR-196-1</i>	AC103702	17q21	46.9–47.1	UAGGUAGUUUCAUGUUGUJGG
<i>miR-181a</i>	AL158075	9q33.1–34.13	120.85–0.95	AACAUUCAACGCUGUCGGUGAGU
<i>miR-223</i>	AL034397	Xq12–13.3	63.4–0.5	UGUCAGUUUGUCAAAUACCC

For each miRNA, genomic position and sequences are shown. Sequences have been taken from the Sanger miRNA Registry at <http://www.sanger.ac.uk/>.

PCR were carried out using the ABI Prism 7700 Sequence Detector System (Applied Biosystems). Total RNA was extracted from a total of 10×10^6 to 20×10^6 thawed cells using Trizol (Invitrogen) purification method. First-strand cDNA synthesis was carried out from 250 ng of total RNA in 7.5 μ l of final volume containing 50 nM stem-loop primer, $1 \times$ RT buffer, 3.33 U/ μ l MultiScribe reverse transcriptase and 0.25 U/ μ l RNase inhibitor. The mix was incubated in a 96 well plate at 16°C for 30 min, 42°C for 30 min, 85°C for 5 min and then held at 4°C. All RT reactions including no-template controls were run in duplicate. Real-time PCR was performed using a standard TaqMan PCR protocol. The 10 μ l PCR reactions included 0.67 μ l of RT product, $1 \times$ Universal TaqMan Master Mix and $1 \times$ TaqMan probe/primer mix. The reactions were incubated in a 96-well plate at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All RT reactions including no-template controls were run in triplicate. Input amount of total RNA samples was previously normalized based on the TaqMan Gene Expression Assay for human glyceraldehyde-3-phosphate dehydrogenase endogenous controls (P/N: 4310884E, Applied Biosystems) as recommended by Chen *et al.*²² (data not shown). *MiR-223* was used as endogenous control. Data were normalized against *miR-223*, uniformly expressed across the set of samples (average C_t for 30 AML samples = 15.08; s.d. of the average C_t = 0.67). The relative amount of transcript was calculated using the comparative C_t method (Supplementary Table S4).

Statistical analysis

Full details of the computational calculations are reported in Supplementary Information. In brief, the Pearson and Spearman correlation coefficients were calculated for each miRNA paired in turn with each gene measured in the AML genome-wide analysis. To make the findings robust to random association, the expression values of each miRNA were randomized among the AMLs and the correlation coefficients recalculated (Supplementary Figure S1). The randomization and recalculation of coefficient distribution was repeated 500 times with the same result (data not shown).

Results and discussion

Previous gene expression profile analysis of AML samples has shown a distinctive up-regulation of members of the HOXA and HOXB gene families in leukaemias with a normal karyotype.²³ Further studies of AMLs with a normal karyotype confirmed and extended the finding, providing evidence for the presence of two distinct groups with different HOX gene expression levels (Debernardi *et al. Blood* 2003; **102**: 1322, abstract). Homeobox genes have been suggested to be among the most represented targets of miRNAs.^{28,33} The levels of five particular miRNAs

(Table 1) were assessed using the ‘looped’ real-time PCR assay, specifically designed to measure quantitatively the mature product,²² across a set of 30 normal karyotype AMLs (full sample details are described in Supplementary Table S1), for which the expression profile was available. The miRNAs were either located within the HOX clusters (*miR-10a*, *miR-10b* and *miR-196a-1*) or known to target homeobox genes^{27–29,34} and to be involved in haematopoietic development (*miR-181a* and *miR-223*).²⁶ *MiR-223*, uniformly expressed across the set of samples, was used to normalize the data. The miRNA assay, which was performed in triplicate, reproducibly detected expression for *miR-181a*, *miR-10a*, *miR-10b* and *miR-196a-1*. (The detailed values are reported in Supplementary Table S4.)

We wished to determine whether the expression of an individual miRNA could be correlated, either negatively or positively, with the expression of any particular gene or group of genes across the set of 30 AMLs. Hence, both the Pearson and Spearman correlation coefficients were calculated for each miRNA paired, in turn, with each of the 45 000 probe sets on the U133 set expression arrays (Affymetrix), representing nearly 39 000 transcripts. To exclude possible chance associations, the expression values of each miRNA were randomized among the samples and the correlation coefficients recalculated. The distribution of correlation coefficients derived from randomized data was Gaussian and more restricted in range than the actual data (Supplementary Figure S1). The heavier tails in the experimental distribution indicated that a significant number of genes exhibited a degree of correlation with each miRNA expression that was greater than would be expected by chance. To identify those genes, the level of significance was chosen to be outside the range of the randomized distribution and above the 99.95% quantile of the experimental distribution for positively correlated genes and below the 0.05% quantile for the negatively correlated genes (Table 2). The most significant positively and negatively correlated genes were identified from the AML microarray expression profile data, with both association measures. (The complete lists of genes and their correlation coefficient values, calculated for *miR-181a*, *miR-10a*, *miR-10b* and *miR-196a-1* are reported in Supplementary Tables S5, S6, S7 and S8, respectively.)

MiR-181a correlates with the expression of its target genes and with the leukaemic morphological sub-type

As reported in Table 2, *miR-181a* showed the highest number of significantly correlated genes. It is notable that 28 genes among these had been previously proposed, purely on computational predictive grounds, to be targets of *miR-181a* (Table 3).^{27–29,34,35} The negative correlation showed by 15 of these 28 genes suggests that *miR-181a* in leukaemic cells may be directly regulating the transcript level of a number of its targets. A more complex model of regulation would be required to explain the

Table 2 Number of the most statistically significant correlated and anti-correlated genes obtained with either the Pearson or the Spearman correlation coefficient

	No. of positively correlated genes	No. of negatively correlated genes	Total
<i>miR181a</i> (Pearson correlation)	112	163	275
<i>miR181a</i> (Spearman correlation)	154	233	387
<i>miR196a-1</i> (Pearson correlation)	47	9	56
<i>miR196a-1</i> (Spearman correlation)	33	6	39
<i>miR10a</i> (Pearson correlation)	19	1	20
<i>miR10a</i> (Spearman correlation)	19	0	19
<i>miR10b</i> (Pearson correlation)	17	2	19
<i>miR10b</i> (Spearman correlation)	13	2	15

The level of significance was chosen to be outside the range of the randomized distribution, and above the 99.95% quantile of the experimental distribution for positively correlated genes and below the 0.05% quantile for the negatively correlated.

Table 3 List of the 28 target genes of *miR-181a* that show statistically significant correlation in the leukaemia samples

<i>miR-181a</i> correlated/target genes	Gene title	Type of correlation	RefSeq transcript ID
AKAP7	A kinase (PRKA) anchor protein 7	neg	NM_004842
BCL2	B-cell CLL/lymphoma 2	pos	NM_000633
BCL2L11	BCL2-like 11 (apoptosis facilitator)	neg	NM_006538
CPEB4	Cytoplasmic polyadenylation element binding protein 4	neg	NM_030627
CPNE2	Copine II	neg	NM_152727
DAZAP2	DAZ-associated protein 2	neg	NM_014764
DLC1	Deleted in liver cancer 1	pos	NM_006094
ETV6	Ets variant gene 6 (TEL oncogene)	pos	NM_001987
FOXP1	Forkhead box P1	pos	NM_001012505
GHITM	Growth hormone-inducible transmembrane protein	neg	NM_014394
GRB10	Growth factor receptor-bound protein 10	pos	NM_001001549
HMGB1	High-mobility group box 1	pos	NM_002128
INPP5E	Inositol polyphosphate-5-phosphatase, 72 kDa	pos	NM_019892
KIAA0182	KIAA0182	pos	NM_014615
KLF3	Kruppel-like factor 3 (basic)	neg	NM_016531
KLHL2	Kelch-like 2, Mayven (Drosophila)	neg	NM_007246
MAP2K1	Mitogen-activated protein kinase kinase 1	neg	NM_002755
MTPN	Myotrophin	neg	NM_145808
NDFIP1	Nedd4 family interacting protein 1	neg	NM_030571
PI4KII	Phosphatidylinositol 4-kinase type II	neg	NM_018425
RIN2	Ras and Rab interactor 2	neg	NM_018993
RKHD3	Ring finger and KH domain containing 3	pos	NM_032246
SLC9A6	Solute carrier family 9, member 6	neg	NM_006359
STMN1	Stathmin 1/oncoprotein 18	pos	NM_005563
STRBP	Spermatid perinuclear RNA binding protein	pos	NM_018387
TGIF2	TGFB-induced factor 2 (TALE family homeobox)	pos	NM_021809
TOX	Thymus high mobility group box protein TOX	pos	NM_014729
ZDHHC7	Zinc finger, DHHC-type containing 7	neg	NM_017740

The type of correlation with *miR-181a* is indicated (neg, negative; pos, positive). The 28 genes were identified by comparison of the correlated gene lists with over 600 reported targets for *miR-181a* at www.targetscan.org,^{27,35} at www.microrna.org²⁸ and obtained with PicTar algorithm.²⁹

positive correlation between *miR-181a* and the remaining 13 targets.

A hierarchical cluster analysis of the AML samples using the set of genes most significantly correlated with *miR-181a*, clearly identified a natural grouping of the data based on the morphological FAB phenotype²⁴ as shown by the dendrogram in Figure 1a. The levels of expression of *miRNA-181a*, shown in Figure 1b, were elevated in samples with M1 or M2 morphology, compared with the samples with M4 or M5 morphology. By contrast, in normal human BM, *miR-181a* has been reported to be preferentially expressed in B cells, T cells, monocytes and granulocytes,³² which are more closely allied to the M4 and M5 morphological leukaemic subtypes. The elevated expression of *miR-181a* observed here in myeloblastic leukaemias implies its involvement in the leukaemic process.

miR-10a, miR-10b and miR-196a-1 correlate with HOX genes

The expression of the three miRNAs located in intergenic regions in the HOX clusters, *miR-10a*, *miR-10b* and *miR-196a-1*, showed a distinctive correlation with that of the HOX genes in the 30 AMLs. The correlation coefficient values were high and positive for almost all the genes of the HOXA and HOXB clusters, with the exception of *HOXA1* and *HOXA13* in cluster A and *HOXB1* and *HOXB13* in cluster B (Supplementary Table S9). By contrast, with the results for human leukaemic tissue presented here, *HOXA1* listed among the putative targets for both *miR-10a* and *miR-10b*, in the K562 myeloblastic cell line, has been experimentally shown to be down-regulated by ectopic expression of *miR-10a*.³⁶ In mouse embryos, it has been shown experimentally that *HOXB8* is target for *miRNA-196a*.^{3,4} Both cases underline the specificity of the

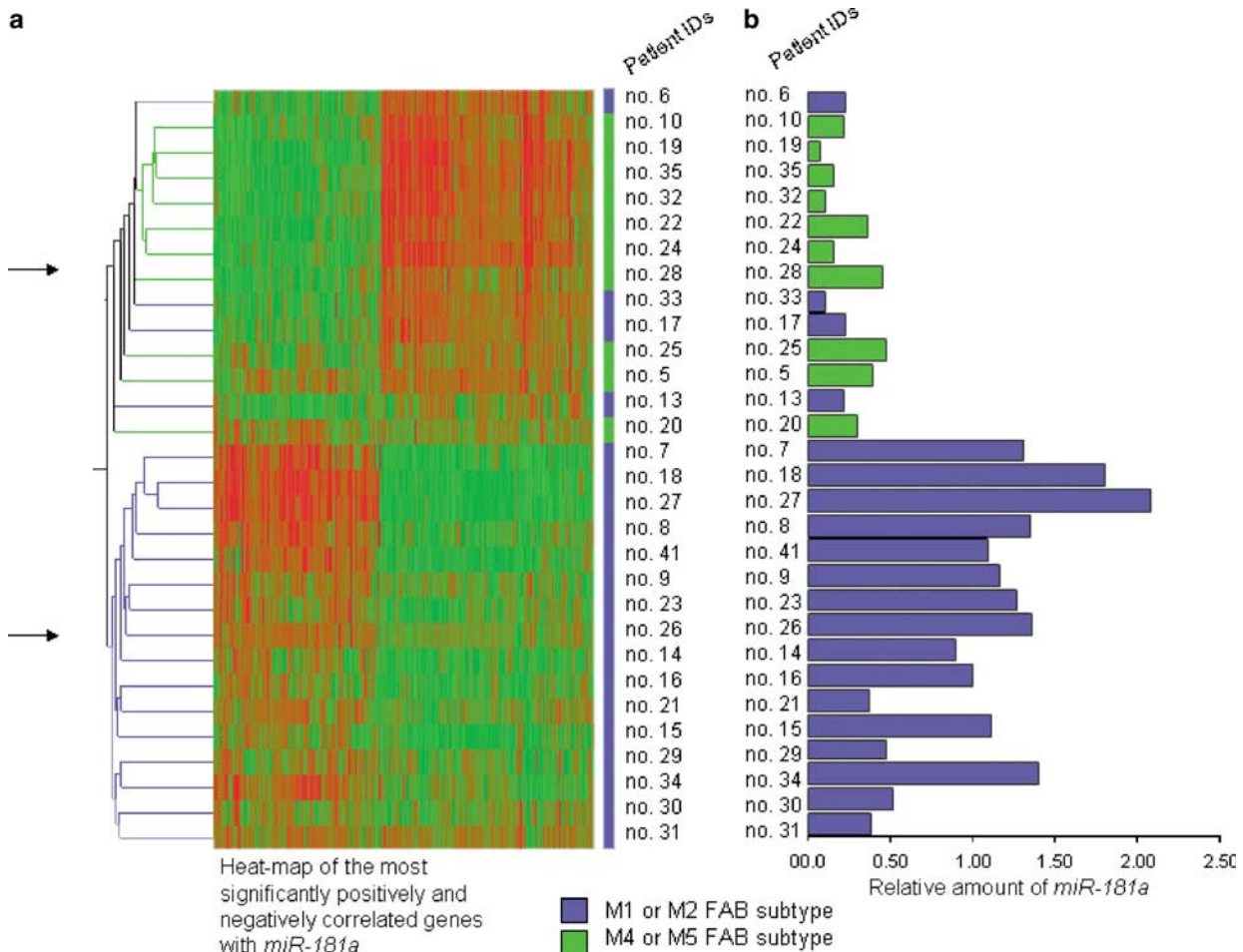


Figure 1 Hierarchical cluster analysis of the 30 normal karyotype AML samples performed with the 387 most significant genes, including both correlated and anti-correlated with *miR-181a* obtained with Spearman correlation. (a) The two-way (genes against samples) hierarchical cluster of the 30 patient samples (rows) and 387 genes with variable level of expression (columns). (b) The quantitative measurement of *miR-181a* for the same samples (horizontal bars). Samples with M1 or M2 morphology are depicted in blue and those with M4 or M5 in green. It is clear that gene expression divided the samples in two major clusters, indicated by the arrows, one including those with M1 or M2 morphology (blue branches) and the other mainly samples with M4 or M5 morphology (green branches). The heat map display encodes the logarithm of the expression changes, where red indicates expression levels greater than the mean and green levels less than the mean.

correlation of expression of HOX genes and miRNAs with species and particular tissue type.

No significant correlation was found with any of the genes of the other two clusters, HOXC and HOXD, whose expression was almost undetectable in the microarray analysis and did not show a modulated profile across the patient set (Supplementary Table S9). Finding an association in expression levels between miRNAs and HOX gene expression does not necessarily imply a direct causal link. On the contrary, the expression of the miRNAs within the HOX clusters could be affected by the same regulatory elements across paralog clusters. It has been reported that in mouse embryos, the two homologues *miR-10a* and *miR-196a* seemed to be expressed in patterns that were reminiscent of those of HOX genes,³ but no reference was made with regard to chromosomal position.

Nearly 30% of the genes showing a high correlation with *miRNA-10a*, *miRNA-10b* and *miRNA-196a-1* have been shown to have oncogenic potential, in addition to the HOX genes (Supplementary Tables S6, S7 and S8). Positive correlation was found for *MEIS1*, *PBX3*, *RUNX1* and *JUND*. The tyrosine kinase oncogene *FES*³⁷ and the pro-apoptotic *FADD* were negatively correlated. The overrepresentation of transcription factors supports

a previous hypothesis of oncogene activation driven by elevated level of HOX genes associated with leukaemogenesis.^{38,39}

Conclusions

This study has established the association of level of *miR-181a* with specific leukaemic phenotype, suggesting a possible role in the developmental lineage and differentiation state of the tumour. Moreover, it has highlighted the correlation of *miR-181a* level with that of a number of its targets, suggesting the direct regulation at transcript level. This study illustrates the potential for using quantitative measurement of miRNA expression to sub-classify cancer and suggests that a more detailed analysis of a larger numbers of miRNAs could provide valuable insights into the leukaemogenic process.

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References

- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281–297.
- Doench JG, Sharp PA. Specificity of microRNA target selection in translational repression. *Genes Dev* 2004; **18**: 504–511.
- Mansfield JH, Harfe BD, Nissen R, Obenaus J, Srineel J, Chaudhuri A et al. MicroRNA-responsive ‘sensor’ transgenes uncover Hox-like and other developmentally regulated patterns of vertebrate microRNA expression. *Nat Genet* 2004; **36**: 1079–1083.
- Yekta S, Shih IH, Bartel DP. MicroRNA-directed cleavage of *HOXB8* mRNA. *Science* 2004; **304**: 594–596.
- Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R et al. Regulation by *let-7* and *lin-4* miRNAs results in target mRNA degradation. *Cell* 2005; **122**: 553–563.
- Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, Chen J et al. Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell* 2005; **120**: 623–634.
- Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A et al. RAS is regulated by the *let-7* microRNA family. *Cell* 2005; **120**: 635–647.
- Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005; **433**: 769–773.
- Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 2004; **101**: 2999–3004.
- Calin GA, Croce CM. MicroRNAs and chromosomal abnormalities in cancer cells. *Oncogene* 2006; **25**: 6202–6210.
- Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E et al. Frequent deletions and down-regulation of micro-RNA genes *miR15* and *miR16* at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002; **99**: 15524–15529.
- Michael MZ, SM OC, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 2003; **1**: 882–891.
- Metzler M, Wilda M, Busch K, Viehmann S, Borkhardt A. High expression of precursor microRNA-155/*BIC* RNA in children with Burkitt lymphoma. *Genes Chromosomes Cancer* 2004; **39**: 167–169.
- Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H et al. Reduced expression of the *let-7* microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* 2004; **64**: 3753–3756.
- Ciafre SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G et al. Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem Biophys Res Commun* 2005; **334**: 1351–1358.
- Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M et al. *miR-15* and *miR-16* induce apoptosis by targeting *BCL2*. *Proc Natl Acad Sci USA* 2005; **102**: 13944–13949.
- Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S et al. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res* 2005; **65**: 7065–7070.
- Pallante P, Visone R, Ferracin M, Ferraro A, Berlingieri MT, Troncone G et al. MicroRNA deregulation in human thyroid papillary carcinomas. *Endocr Relat Cancer* 2006; **13**: 497–508.
- Roldo C, Missiaglia E, Hagan JP, Falconi M, Capelli P, Bersani S et al. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. *J Clin Oncol* 2006; **24**: 4677–4684.
- Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006; **9**: 189–198.
- Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D et al. MicroRNA expression profiles classify human cancers. *Nature* 2005; **435**: 834–838.
- Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT et al. Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Res* 2005; **33**: e179.
- Debernardi S, Lillington DM, Chaplin T, Tomlinson S, Amess J, Rohatiner A et al. Genome-wide analysis of acute myeloid leukemia with normal karyotype reveals a unique pattern of homeobox gene expression distinct from those with translocation-mediated fusion events. *Genes Chromosomes Cancer* 2003; **37**: 149–158.
- Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. *Br J Haematol* 1976; **33**: 451–458.
- Griffiths-Jones S. miRBase: the microRNA sequence database. *Methods Mol Biol* 2006; **342**: 129–138.
- Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science* 2004; **303**: 83–86.
- Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. *Cell* 2003; **115**: 787–798.
- John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA Targets. *PLoS Biol* 2004; **2**: e363.
- Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ et al. Combinatorial microRNA target predictions. *Nat Genet* 2005; **37**: 495–500.
- Lagos-Quintana M, Rauhut R, Meyer J, Borkhardt A, Tuschl T. New microRNAs from mouse and human. *RNA* 2003; **9**: 175–179.
- Lim LP, Glasner ME, Yekta S, Burge CB, Bartel DP. Vertebrate microRNA genes. *Science* 2003; **299**: 1540.
- Ramkissoon SH, Mainwaring LA, Ogasawara Y, Keyvanfar K, McCoy Jr JP, Sloand EM et al. Hematopoietic-specific microRNA expression in human cells. *Leuk Res* 2006; **30**: 643–647.
- Smalheiser NR, Torvik VI. A population-based statistical approach identifies parameters characteristic of human microRNA-mRNA interactions. *BMC Bioinformatics* 2004; **5**: 139.
- Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* 2006; **34**: D140–144.
- Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; **120**: 15–20.
- Garzon R, Pichiorri F, Palumbo T, Iuliano R, Cimmino A, Aqeilan R et al. MicroRNA fingerprints during human megakaryocytopoiesis. *Proc Natl Acad Sci USA* 2006; **103**: 5078–5083.
- Scheijen B, Griffin JD. Tyrosine kinase oncogenes in normal hematopoiesis and hematological disease. *Oncogene* 2002; **21**: 3314–3333.
- Thorsteinsdottir U, Sauvageau G, Hough MR, Dragowska WH, Lansdorp P, Lawrence HJ et al. Overexpression of *HOXA10* in murine hematopoietic cells perturbs both myeloid and lymphoid differentiation and leads to acute myeloid leukemia. *Mol Cell Biol* 1997; **17**: 495–505.
- Thorsteinsdottir U, Mamo A, Kroon E, Jerome L, Bijl J, Lawrence HJ et al. Overexpression of the myeloid leukemia-associated *Hoxa9* gene in bone marrow cells induces stem cell expansion. *Blood* 2002; **99**: 121–129.

Supplementary Information accompanies the paper on the Leukemia website (<http://www.nature.com/leu>)