Quantitative Measurement of microRNAs Reveals Distinct Correlation with Acute Myeloid Leukemia (AML) Signature

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INTRODUCTION

MicroRNAs (miRNAs) are short single stranded RNAs that have a potentially important role in gene regulation. That role is due to complementarity mediated binding to target mRNAs resulting in the repression of translation and in the cleavage of the target transcript. There are indications that miRNAs might be a new class of genes involved in human tumorigenesis.

Using a novel quantitative real-time PCR assay, specific to the mature product, the expression level of a selected group of miRNAs was measured across a set of 30 primary adult acute myeloid leukemia (AML) with a normal karyotype. Calculation of the correlation between miRNA levels and the genome-wide AML expression profile identified distinct patterns of miRNA expression that correlate with the AML signature. In addition, the genes whose expression was most significantly associated with each miRNA were identified. This is the first study reporting a quantitative measurement of mature miRNA expression and the computational discovery of genome-wide associations in primary malignancies.

METHODS

MicroRNAs were quantitated by real-time PCR using TaqMan® -MicroRNA assay (Applied Biosystems)* in a set of 30 acute myeloid leukaemias from 250 ng of total RNA. Ten miRNAs were selected from the Sanger miRNA Registry at http://www.sanger.ac.uk/Software/Rfam/mirna/index.shtml (Table 1).

Name	Chr location	GenBank	miRNA Loc (Mb)	Mature miR
miR-10a	17q21.3	AF287967	46.95-47.05	UACCCUGUAGAUCCGAAUUUGUG
miR-10b	2q31	AC009336	176.85-177	UACCCUGUAGAACCGAAUUUGU
miR-196-1	17q21	AC103702	46.9-47.1	UAGGUAGUUUCAUGUUGUUGG
miR-196-2	12q13	AF490843	54-54.15	UAGGUAGUUUCAUGUUGUUGGG
miR-148	7p15	AC010719	25.6-8	UCAGUGCACUACAGAACUUUGU
miR-152	17q21	AC004477	46.4-5	UCAGUGCAUGACAGAACUUGG
miR-19a	13q31	AL162375	90.82	UGUGCAAAUCUAUGCAAAACUGA
miR-181a	9q33.1-34.13	AL158075	120.85-.95	AACAUUCAACGCUGUCGGUGAGU
miR-181c	19p13.3	AC020916	13.75-.95	AACAUUCAACCUGUCGGUGAGU
miR-223	Xq12-13.3	AL034397	63.4-.5	UGUCAGUUUGUCAAAUACCCC

The measurement included 6 miRNAs located within the HOX clusters (Figure 1) (*miR-10a* and *miR-196a-1* on 17q21, *miR-196a-2* at 12q13, *miR-10b* at 2q31, and *miR-148* and *miR-152* located within 1 Mb from the HOX clusters at 7p15 and 17q21, respectively) and 4 miRNAs known to be involved in haematopoietic development, or having as targets homeobox genes *(miR-181a, miR181c, miR-223*, and *miR-19a*).

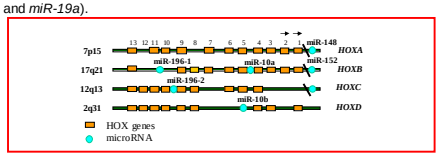

Figure 1. MiRNAs located in the human HOX clusters.

Data were normalised against *miR-223*, equally expressed across the set of samples (Ave. Ct for 30 AML samples = 15.08; Std. Dev. of the Ave. Ct = 0.67) (Figure 2). The relative amount of all the miRNAs transcript was calculated using the comparative Ct method.

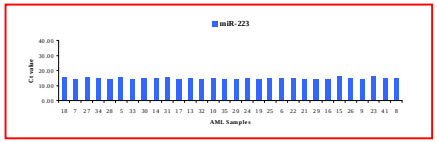

Figure 2. *MiR-223* was used as endogenous control.

* The assays, which are specific to the mature miRNAs and do not detect the precursor species, were provided as part of a collaboration agreement with Applied Biosystems (www.appliedbiosystems.com).

RESULTS

1. Summary of the miRNA quantitation results

miR-10a ***miR-10b*** ***miR-196a-1*** ***miR-196a-2***	**Expressed and showing variability across the sample set**
miR-148a ***miR-152***	**Not expressed**
miR-181a ***miR-181c***	**Expressed and showing variability across the sample set**
miR-19a	**Expressed with no variation across the set**

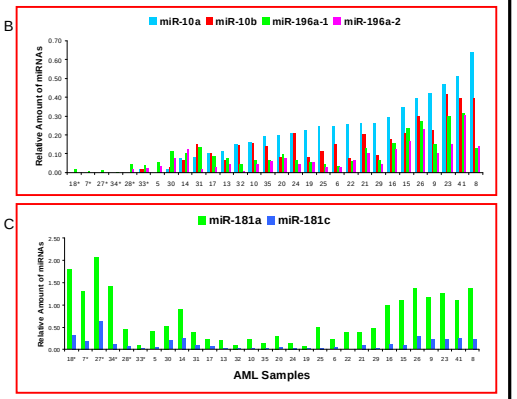

Figure 3. Summary of the real-time PCR results (panel A). Relative amount of transcript quantitatively measured of *miR-10a*, *miR-10b*, *miR-196a-1*, and *miR-196a-2* (panel B), and *miR-181a* and *miR-181c* (panel C), calculated for 30 AML samples. (* indicates the 6 samples with low expression of certain HOX genes as detected from expression profile and real time PCR experiments).

2. Correlation between expression level of microRNAs and HOX genes

To measure the strength of linear association between miRNAs and HOX genes, the Pearson correlation coefficient was calculated for each of the 4 miRNAs (*miR-10a*, *miR-10b*, *miR-196a-1*, and *miR-196a-2)*, and each of the 39 HOX genes of the four clusters measured in the microarray experiments (Figure 4).

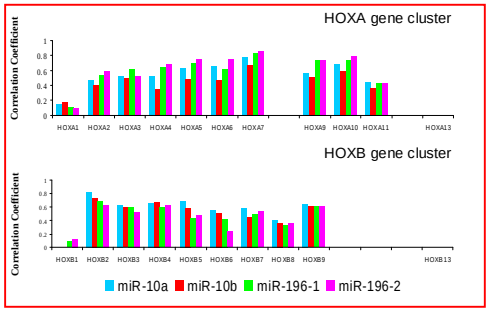

Figure 4. The Pearson correlation coefficients calculated for each miRNA and each HOX gene. A) Correlation between *miR-10a*, *miR10b*, and *miR-196a-1* and HOXA cluster genes on 7p15. B) Correlation between *miR-10a*, *miR10b*, and *miR-196a-1* and HOXB cluster genes on 17q21. HOX genes are positioned to match their chromosomal organisation.

3. Association between expression level of microRNAs and AML genome-wide expression quantitatively measured using the Pearson Correlation Coefficient

To capture the general effect of the expression of miRNAs on the AML signature.For each miRNA [*miR-10a*, *miR-10b*, *miR-196a-1*, and *miR-196a-2*, in the HOX clusters, and of *miR-181a*] correlation and anti-correlation was calculated with each gene measured in the AML genome-wide analysis.

To make the findings robust to random association, randomly generated miRNA experiments, were used to identify the level of the correlation coefficient considered as significant (Figure 5).

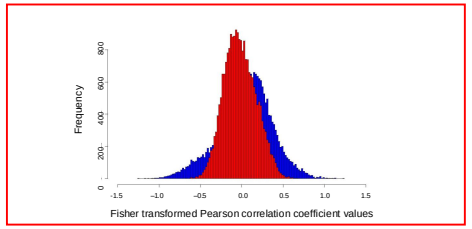

Figure 5. Histogram of Fisher transformed Pearson Correlation Coefficient distribution of *miR-181a* and gene expression of AMLs, for the experimentally acquired values(**blue**) and the ones randomly generated (**red**). The level of significance was chosen to be outside the range of the randomised distribution, and above the 99.95% quantile of the experimental distribution for correlated genes and below 0.05% for the anti-correlated.

Most significant genes

miRNA	n. Pos. Corr. Genes	n. Neg. Corr. Genes
miR-10a	*20 +*	*1 -*
miR-10b	*19 +*	*1-*
miR-196a-1	*47 +*	*9 -*
miR-196a-2	25 +	8 -

Nearly 30 % of these genes were known to have oncogenic potential. Besides the HOX genes, positive correlation was in fact found for *MEIS1*, *PBX3*, *RUNX1*, and *JUND.* Anti-correlated were the tyrosine kinase *FES* and the pro-apoptotic *FADD.*

miRNA	n. Pos. Corr. Genes	n. Neg. Corr. Genes
miR-181a	154 +	234 –

Association of an elevated intracellular amount of the mature *miR-181a* transcript with the silencing of several members of the Rho family of small G proteins, such as *RAB31*, *RHOQ*, and *RAC1* and a number of kinases, i.e. *PAK1*, *MAP2K1*, and *PRKCA* involved in the integrin-mediated signal transduction pathway (Figure 6). Association with the activation of genes involved in the ubiquitin pathway. A number of the genes identified are known target of *miR-181a* (thus confirming computationally predicted published results).

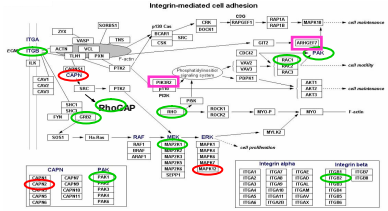

Figure 6. Integrin-mediated signal transduction pathway. Genes correlated and anti-correlated with *miR-181a* are circled in red and in green, respectively.

4. Correlation between expression level of *miR-181a* and AML genome-wide expression

A hierarchical cluster analysis of the 30 AML samples using the genes most significantly associated with *miR-181a*, clearly identified a natural grouping of the data based on the morphological FAB phenotype, as shown by the dendrogram in Figure 7A. The levels of expression of *miRNA-181a* are shown in Figure 7B.

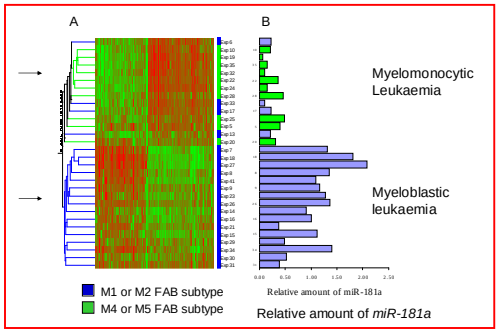

Figure 7. Hierarchical cluster analysis of the 30 AML samples performed with the 388 genes, including both the correlated and the anti-correlated with *miR-181a*. Panel A shows the two-way (genes against samples) hierarchical cluster of the 30 samples (rows) and 388 genes with variable level of expression (column). Panel B shows the quantitative measurement of *miR-181a* for the same samples (horizontal bars). Samples with M1 or M2 morphology are depicted in blue, those with M4 or M5 are in green.

CONCLUSIONS

The development of a novel quantitative "looped" real-time PCR assay (Applied Biosystem) for the mature miRNA expression levels has allowed us to demonstrate, using computational methods, that particular miRNA levels are associated with gene expression profiles in primary adult acute myeloid leukemia.

miR-10a, *miR-10b*, *miR-196a-1*, and *miR-196a-2* molecules showed a correlation with the expression of certain HOX genes and of a number of oncogenes, suggesting that they may have a role in the leukemogenic process of AML with a normal karyotype.

miR-181a strongly correlated with specific leukemic phenotype, suggesting a possible role in haematopoietic differentiation and in determining cell fate, raising the hypothesis of a different clonal origin of myeloblastic and monoblastic leukaemia.

This work 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 leukemogenic process.

Acknowledgements

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