

## Silencing of *hsa-miR-124* by *EVI1* in cell lines and patients with acute myeloid leukemia

We read with great interest the work published by Dickstein et al. (1) showing that induced *EVI1* expression in a murine model silences *miR-124* expression by DNA methylation. The *EVI1* gene codes for a transcription factor implicated in the development and progression of high-risk acute myeloid leukemia (AML) (2, 3). We quantify the expression of 250 microRNAs (miRNAs; TaqManHuman miRNA assay set) in 15 myeloid cell lines. Statistical analysis comparing cell lines with and without *EVI1* protein identified miRNAs differentially expressed ( $B > 0$ ). Among them, cell lines with *EVI1* protein had no expression of *hsa-miR-124*, whereas most cell lines with no *EVI1* had high *hsa-miR-124* expression (Table 1). Interestingly, MEG-01 cells, with *EVI1* overexpression and no protein, expressed low levels of *hsa-miR-124* (Fig. 1 and Table 1). We first considered whether *EVI1* was a target of *hsa-miR-124*. Transiently, transfection of pre-*hsa-miR-124* in HEL and KU-812 cell lines, both with *EVI1* protein and no *hsa-miR-124* expression, showed a dramatic increase of *hsa-miR-124*; however, no changes in *EVI1* expression either at the mRNA or protein level were detected. These results indicate that *hsa-miR-124* does not regulate *EVI1* expression. The results of Dickstein et al. (1) prompted us to examine whether DNA methylation could be responsible for the low expression of *hsa-miR-124* in cell lines with *EVI1* protein (1). We analyzed the methylation status of *hsa-miR-124-1* by methylation-specific PCR as previously described (4). All of the cell lines analyzed that had low expression of *hsa-miR-124* had the promoter methylated: four had *EVI1*, and three had no protein. Conversely, the two cell lines with high expression of *hsa-miR-124* had no methylation and no *EVI1* (Table 1). These results strongly support the hypothesis that *EVI1* silences *hsa-miR-124* expression by DNA methylation (1), although they would also indicate that, in some cases, the expression of *hsa-miR-124* might be regulated by other mechanisms. To check the clinical importance of these results, we analyzed 42 AML patients, 19 of which had *EVI1* overexpression (Table 1). Consistent with our results in cells lines, expression of *EVI1* in patients was associated with decreased expression of

*hsa-miR-124* ( $P = 0.036$ ), supporting that *EVI1* could play a role in the transcriptional regulation of *hsa-miR-124* (Fig. 1). Nevertheless, as in cell lines, some cases with low *hsa-miR-124* expression had no *EVI1* overexpression.

Further studies are needed to fully clarify the role of *hsa-miR-124* silencing in *EVI1*-positive AML. As shown in other malignancies, cyclin-dependent kinase 6 (CDK6) could be a target of *hsa-miR-124* in AML. In the HEL cell line (*hsa-miR-124-1*-methylated and CDK6-expressed), we found that CDK4/6 inhibition by PD-0332991 induced dephosphorylation of retinoblastoma (Rb), with no changes in the protein levels of Rb or CDK6, and inhibited cell growth. These results may provide alternatives for future AML treatment. In conclusion, our results support the findings of Dickstein et al. (1), suggesting that a common mechanism for *hsa-miR-124* down-regulation in hematological malignancies is the methylation of the promoter region of this gene and that *EVI1* has a role in the transcriptional regulation of *hsa-miR-124* expression.

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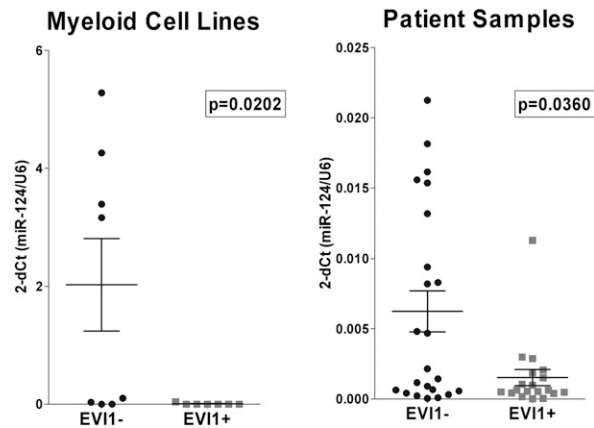


Fig. 1. Diagram showing the expression of *hsa-miR-124* in 15 myeloid cell lines and 42 samples of patients with AML. Data represents the mean  $\pm$  SE.

Table 1. Quantification of the expression of *hsa-miR-124* and methylation status of the *hsa-miR-124-1* promoter region in 15 human myeloid cell lines

Cell line	Diagnosis	<i>hsa-miR-124</i> (2-dct)	<i>hsa-miR-124-1</i> (methylation status)	Evi1 protein	Overexpression (qRT-PCR)					
					EVI1-1A	EVI1-1B	EVI1-1C	EVI1-1D	EVI1-3L	MDS1EVI1
MV4-11	AML-M5	4.2575	UM	No	No	No	No	No	No	No
NOMO-1	AML-M5	3.3870	UM	No	No	No	No	No	No	No
MOLM-13	AML-M5	3.1602	nd	No	No	No	No	No	No	No
OCI-AML2	AML-M4	5.2780	nd	No	No	No	No	No	No	No
MEG-01	CML-BP	0.1022	nd	No	No	Yes	No	No	No	No
KG-1	AML-M6	0.0337	M	No	No	No	No	No	No	No
Kasumi-1	AML-M2	0.0000	M	No	No	No	No	No	No	No
HL-60	AML-M2	0.0000	M	No	No	No	No	No	No	No
MUTZ-3	AML-M4	0.0398	M	Yes	Yes	Yes	Yes	Yes	No	No
KU-812	CML-BP	0.0009	nd	Yes	No	Yes	No	No	No	No
TF-1	AML-M6	0.0001	M	Yes	Yes	Yes	Yes	Yes	No	No
HEL	AML-M6	0.0001	M	Yes	Yes	Yes	Yes	Yes	No	No
K562	CML-BP	0.0001	nd	Yes	Yes	Yes	No	No	No	Yes
F-36P	AML-M6	0.0001	M	Yes	Yes	Yes	Yes	Yes	No	No
KYO-1	CML-BP	0.0001	nd	Yes	No	Yes	Yes	No	No	No

CML-BP, chronic myeloid leukemia blast phase; M, methylated; nd, no data; UM, unmethylated.