MiR-125b and miR-99a encoded on chromosome 21 co-regulate vincristine resistance in childhood acute megakaryoblastic leukemia

To the Editor: MicroRNAs (miRNAs) are small, non-coding RNA of 20–22 nucleotides in length which can silence specific target genes through direct mRNA degradation, translational repression, or both. miRNAs are involved in several cellular processes like cell cycle, apoptosis, drug resistance and differentiation. In 2011, Schotte et al. reported different miRNA signatures in pediatric acute lymphoblastic leukemia (ALL) with various genetic subtypes and drug-resistance. miR-125b, miR-99a, and miR-100 were upregulated ~20-fold in vincristine and daunorubicin-resistant ALL cells. Recent reports suggested that co-expression of miR-125b in combination with miR-100, or miR-99a, or miR-100 and miR-99a induces significant vincristine (VCR) resistance in ET6-V6-RUNX1-positive Reh leukemic cells. The authors noted that the miR-125b isoforms, (miR-125b-1 and miR-125b-2), were located downstream to miR-100 and miR-99a, respectively. Also, both miR-100 and miR-99a differed by a single nucleotide. Therefore, co-expression of miR-125b/miR-99a, miR-125b/miR-100 or miR-125b/miR-99a/miR-100 is required for VCR resistance.

A growing body of evidence suggests the crucial role of miRNAs in the regulation of differentiation in normal as well as malignant hematopoiesis. Screening of miRNA for this drug resistance is needed to estimate the prognosis of the disease and to identify better drug targets. Five miRNAs (miR-99a, let-7c, miR-125b-2, miR-155 and miR-802) are encoded on chromosome 21, and are potential players in the pathogenesis of transient myeloproliferative disorder (TMD). TMD of Down syndrome is a hematologic abnormality which is characterized by an uncontrolled proliferation of myeloblasts in bone marrow and peripheral blood that affects babies with trisomy of chromosome 21. Interestingly, four of the five miRNAs encoded in chromosome 21 (miR-99a, let-7c, miR-125b-2, and miR-155) play a crucial role in megakaryocyte development. Our laboratory is studying the miRNA expression level of miR-125b and miR-99a in VCR resistant and developmentally different CMK (child) and MEG-01 (adult) megakaryoblastic leukemia cell lines. MEG-01 cell line was established from the bone marrow of a patient with blast crisis of Philadelphia chromosome-positive chronic myelogenous leukemia, and VCR showed no activity against these cells. To show variation in VCR resistance, the cell lines were grown in RPMI 1640 (GIBCO BRL, Grand Island, NY, USA), media supplemented with 10% fetal bovine serum and 1% antibiotics. miRNA (n = 3) was prepared using the miRNeasy mini kit (Qiagen), expression analysis of miR-99a was determined using qRT-PCR, and the data was analyzed by Ct method. Putative targets of miR-125b and miR-99a were explored using bioinformatic tools such as TargetScan, PicTar, RNAhybrid, and miRBase. A confirmatory western blot analysis of the targets was performed by standard method.

First, we compared miR-99a levels in CMK and MEG01 by quantitative PCR and found 8.5-fold upregulation in CMK compared to MEG01 (p < 0.01, n = 3) (Fig. 1A). Our previous report had shown overexpression of miR-125b by nearly 7.5-fold (p < 0.05, n = 3) in CMK cells compared to MEG-01 cells. We found that miR-125b downregulates p53, BAK1 and CDK6 expression. High expression of miR-125b has previously been correlated with drug resistance in leukemia. Previous studies and the current literature show that miR-99a and miR-100 are overexpressed in childhood acute myeloid leukemia (AML). In contrast, miR-99a and miR-100 were downregulated in childhood ALL patients, and their expression levels were related to the prognosis of ALL patients. But the current study is the first report of involvement of miR-125b and miR-99a in VCR resistance of the megakaryoblastic leukemia cell line, CMK.

The clarification of miRNA targets remains a major issue in the functional examination of miRNAs. Putative targets of miR-99a using bioinformatics tools were found to be FKBP51 and IGF1R (Fig. 2). A recent study shows that deregulation of microRNA-100/99a suppresses proliferation and promotes apoptosis by regulating the FKBP51 and IGF1R/mTOR signaling pathways in ALL. The study provided evidence that microRNAs such as miR-99a and miR-100 act as tumor suppressors in ALL cell lines.

Based on these results, we performed protein expression studies of FKBP5 along with IGF1R in CMK and MEG01 cells. We
found a ~0.5-fold downregulation in CMK compared to MEG01 \((p < 0.01, n = 3)\) of FKBP5 and IGF1R protein levels by western blot analysis (Fig. 1B). Increased miR-99a activity resulted in decreased expression of FKBP51 and IGF1R by binding to their mRNA, leading to translational arrest and causing increased p-AKT Ser473 in CMK cells (Figs. 1B and 2). Recently, immunophilin FKBP51 (also known as FKBP5) was identified as a scaffolding protein that can enhance PHLPP-AKT interaction and facilitate PHLPP-mediated dephosphorylation of AKT-Ser473. Downregulation of FKBP51 results in decreased PHLPP-AKT interaction and increased AKT phosphorylation. Our findings in VCR-resistant acute megakaryoblastic leukemia cell line (CMK) derived from Down syndrome patients have shown an overexpression of miR-99a regulating its targets FKBP5 and IGF1R.

The present study suggests that the co-expression of miR-125b and miR-99a contributed to the VCR resistance to CMK cells due to actively transcribed miR99a/miR-125b locus on chromosome 21q21.1. Since miR-99a and miR-125b are located on chromosome 21, an additional copy in Down syndrome correlates to the overexpression of these miRNAs and co-expression of these leads to VCR resistance. Further studies to establish the role of these miRNAs as a therapeutic strategy in leukemia need to be explored.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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