Malignant blasts in acute myeloid leukemia (AML) are organized in a hierarchal manner resembling normal hematopoiesis, with leukemic stem cells (LSCs) at the apex, giving rise to more differentiated progeny with reduced capacity to self-renew (1). Functionally, LSCs demonstrate the ability to propagate disease upon transplantation (demonstrating leukemia-initiating cell, or LIC, activity), as well as serial transplantation. With each transplantation, LSCs cells are capable of retaining properties of the original leukemia, which reflects their unique capacity to self-renew, similar to normal hematopoietic stem cells (HSCs). Clinically, LSCs are thought to mediate disease relapse and chemoresistance, but few validated LSC-targeted therapies have been successfully translated to the clinic (2).

To identify key regulators of LSC function, several groups have performed gene-expression analyses on LSC-enriched cells from AML patient samples (3-5). These studies have demonstrated that LSCs share a similar gene expression signature to HSCs, and that gene signatures from these populations can reliably prognosticate clinical outcomes in normal karyotype AML (3). While several microRNAs (miRNAs), including miR-125a/b and miR-29a, have been shown to maintain HSCs (4,6-8), studies demonstrating functional roles for miRNAs in LSC function in AML are limited (9).

Recently, John Dick and colleagues identified miR-126 as a regulator of LSC function (10). Considering functional LSCs may exist within different immunophenotypically defined leukemia blast populations (11,12), the authors initially sought to identify miRNAs highly associated with LIC activity and therefore fractionated total leukemic blasts into 4 populations based on the presence or absence of CD34 and CD38 surface expression. Subsequently, each population was assessed for LIC activity using xenotransplantation assays. They performed miRNA expression profiling studies on the same populations (10) and identified several miRNAs enriched in the experimentally validated LIC-enriched fractions, including one miRNA known to regulate HSC function (miR-125) (6,8). Of note, miR-99, another miRNA shown to be highly expressed in HSCs (6), displayed the highest level of enrichment in LSCs. We have recently shown that miR-99 plays a critical role in maintaining LSCs in both primary mouse and human AML (manuscript in preparation), which attests to the robustness of the LSC miRNA signature. Additional miRNAs included in the LIC signature included miR-155, which is compatible with previous publications suggesting it serves as an oncogene in AML (13), as well as miR-126, an unexpected finding given that the same group previously demonstrated that it is a negative regulator of HSC function (4).

The signature was evaluated in an AML patient cohort and shown to prognosticate overall survival in a univariate analysis, providing additional support for a role for miR-126 in LSC function. A more recent study corroborated these findings, demonstrating that higher miR-126 levels predict poor overall survival in older AML patients (>60 years) with normal karyotype (14).

As miR-126 had been previously shown by the same group to regulate HSCs (4) and others had shown a potential role in LSCs (4,15), the authors sought to explore the functional role of miR-126 in LSCs. The investigators devised a lentiviral GFP reporter system to assess for biologically active miR-126 in patient AML blasts. Using this system, the authors showed that miR-126 activity is enriched in LIC-enriched populations. However, the correlation between repression of the miR-126 reporter and LIC activity could not be clearly established in all AML...
samples tested, suggesting that miR-126 may not regulate LIC activity in all AML subtypes.

To circumvent the technical limitations of working with primary AML blasts, the authors took advantage of a cell line they established from an AML patient (referred to as 8227) that exhibited several important properties resembling primary AMLs, including a hierarchal organization with CD34+CD38- blasts being the most primitive and exhibiting the highest LSC activity. miR-126 overexpression (OE) in 8227 cells resulted in decreased proliferation, with CD34+CD38- blasts exhibiting increased quiescence as evidenced by decreased BrdU incorporation. In addition, about half the miR-126 OE AML samples exhibited decreased CD15+ differentiation in vitro, and xenotransplantation assays utilizing three primary AML samples transduced with miR-126 OE showed an increased LIC frequency observed in secondary transplantations, establishing miR-126’s role as a positive regulator of LSC activity. In contrast, miR-126 knockdown (KD) using a “sponge” strategy induced increased cycling among CD34+CD38- blasts, enhanced clonogenicity, and induced a small degree of differentiation in primary samples in vitro, but engraftment of miR-126 KD blasts in primary and secondary xenotransplant assays was not impaired. While this may have been due to technical difficulties including the degree of miR-126 KD or the particular samples tested, the authors did not comment on these observations, and therefore the explanation for this finding remains unclear. Follow-up studies using larger number of samples with attempts to correlate outcomes to molecular/cytogenetic subsets would help improve our understanding of the role of miR-126 in LSC function.

The authors next attempted to identify downstream effectors of miR-126 function. As they previously showed that miR-126 inhibits various proteins within the PI3K-AKT-mTOR axis in HSCs (16), they investigated this pathway in LSCs. Mass spectrometry analysis and gene expression profiling in 8227 cells following miR-126 OE and KD identified all three isoforms of AKT and CDK3 (a well-known downstream target of PI3K-AKT) as potential targets of miR-126. As cyclin C forms a complex with CDK3 to promote cell cycle entry (17,18), this target could explain the effects of miR-126 on LSC quiescence. To test whether miR-126 KD effects on LSCs are mediated through induction of CDK3, the authors sought to determine whether CDK3 is necessary and/or sufficient to mimic miR-126 KD effects. While wild type CDK3 OE induced increased proliferation and clonogenicity of both CD34+CD38- and CD34+CD38+ blasts, mutant CDK3 did not, thereby confirming the functional significance of CDK3 kinase activity. As additional cyclin C-CDK complexes influence important LSC-associated signaling pathways mediated by Notch and Wnt (19), it would be interesting to determine whether these pathways mediate miR-126’s effects on LSC self-renewal. It is not clear whether or not concurrent mRNA expression data was generated for the blast populations evaluated in this study; however, the same group did generate a mRNA signature for LSCs (3), which may be useful to identify miR-126 targets since such targets might display inverse expression patterns compared to miR-126 in LSCs vs. non-LSCs.

As chemoresistance in AML is associated with quiescence/dormancy of LSCs (2) and miR-126 OE induced reduced cell cycling, the authors tested whether or not miR-126 OE may confer resistance to standard AML chemotherapies (daunorubicin and cytarabine). The authors not only showed that miR-126 OE confers resistance to 8227 cells as predicted, but that overexpression of CDK3 partially reverses resistance. To further support miR-126’s potential importance in mediating therapy responses, blasts from refractory patients following induction chemotherapy showed higher expression of miR-126. Overall, these data underscore miR-126’s role as a mediator of chemoresistance through its direct inhibition of CDK3 and arrest of G0-G1 exit.

Developing stem cell-directed therapies in AML is challenging given that LSCs share many determinants of stemness with normal HSCs (3). Thus, the studies by Dick and colleagues demonstrating divergent roles for miR-126 in LSC and HSC function credential miR-126 as a candidate therapeutic target in AML. Others have recently investigated the potential of targeting miR-126 in LSCs using nanoparticles (14). After observing that AML patients with normal cytogenetics and relapsed/refractory disease express higher levels of miR-126 in LSC-enriched cells, the investigators used nanoparticles to deliver antagonirs against miR-126. Targeting miR-126 in primary human AML depleted quiescent CD34+ blasts, and loss of LSC activity was demonstrated by decreased self-renewal and increased survival in secondary recipients of miR-126 antagonir-treated CD34+ AML cells. Moreover, the treatment had no negative effects on mouse HSC reconstitution potential, suggesting a potential therapeutic window. To further test the possibility of therapeutically targeting miR-126 in vivo, the authors treated Mll<sup>ITD/ITD</sup> Flt3<sup>ITD/ITD</sup> double knockin mice with their antagonimiR strategy. Treatment resulted in improved...
survival similar to their xenotransplant experiments. While this result was more robust than those observed with the experiments of Dick and colleagues, this may have been due to differences in the extent of gene silencing using of a lentiviral ‘sponge’ strategy versus a traditional antagomir strategy. Understanding the basis of this difference will be important for potential future development of therapies targeting miR-126.

In summary, studies of miR-126 highlight its unique functional roles in AML LSCs and normal HSCs. Given its profound impact on LSC function and its contribution to chemoresistance, partly by reactivation of the PI3K-AKT-CDK3 axis, the data support the development of therapies that target miR-126 or its downstream targets. As translation of antagonim therapeutic strategies remains difficult (20) and given miR-126’s potential importance as a therapeutic target in AML, future studies can help identify cis-acting elements and transcription factors that regulate miR-126 expression, as these may become potential alternative therapeutic targets in the future.

Acknowledgements

Funding: This work was supported by NIH/NCI Grant 1 R01 CA164120-01A1 to C.Y.Park.

Footnote

Provenance: This is an invited Commentary commissioned by the Section Editor Lichao Sun (State Key Laboratory of Molecular Oncology, National Cancer Center (NCC)/ Cancer Hospital, Chinese Academy of Medical Sciences (CAMS), Peking Union Medical College, Beijing, China). Conflicts of Interest: The authors have no conflicts of interest to declare.


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Cite this article as: Elias HK, Khalaj M, Park CY. Divergent roles of miR-126 in normal and malignant stem cells. Transl Cancer Res 2016;5(S2):S328-S331. doi: 10.21037/tcr.2016.07.15