Editorial

Targeting miR-155 in FLT3-ITD mutated AML: ready for prime time?

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The most common genetic aberration in AML is a gain-of-function mutation in the FMS-like tyrosine kinase 3 (FLT3) receptor, which is present in about 30% of CN-AML and confers a poor prognosis (1). FLT3 encodes a receptor tyrosine kinase expressed on hematopoietic progenitor cells involved in stem cell differentiation and proliferation (2). FLT3 activating mutations such as internal tandem duplication (ITD) lead to constitutive, ligand-independent activation of this receptor, conferring a growth and survival advantage. The mutation itself has not been shown to independently drive leukemic transformation *in vivo* (1,3). Rather, FLT3-ITD must collaborate with additional oncogenic mutations to trigger hematopoietic malignancy (3). Despite extensive research throughout the last decades, only Midastaurin has been recently approved by the FDA as a first line treatment in combination with chemotherapy (4) highlighting the difficulties for establishing targeted therapies. Therefore, novel approaches such as targeting downstream effectors of FLT3-ITD signaling are relevant to explore new therapeutic targets.

MicroRNAs (miRNAs) are small noncoding RNAs that control post-transcriptional gene expression in various biological and pathological processes. MiRNA expression has been shown to be highly dysregulated in AML. Specifically, miR-155 is the most significantly overexpressed miRNA in FLT3-ITD mutated AML (5-7). MiR-155 is a known oncogene that accelerates formation of lymphomas and when overexpressed in HSPCs leads to a myeloproliferative disorder as previously shown by O'Connell *et al.* (8,9). Our group recently linked miR-155 upregulation to *MLL*-rearranged AML (10), a subgroup with high FLT3 levels (2) and not only, as previously reported, to FLT3-ITD positive AML (5,7,11) or FAB M4/5 AML (12), implying a broader role for this miRNA in AML. This finding is in line with previous reports in B-cell lymphomas, where miR-155 overexpression was detected in all screened subtypes, regardless of cytogenetics (13). Additionally, high miR-155 expression levels were associated with an inferior overall survival in CN-AML (14) and were found to be part of a leukemic stem cell miRNA signature (15). Enforced expression of miR-155 in myeloid cells has been shown to have both oncogenic and tumor suppressor functions in AML. Palma *et al.* proposed an anti-leukemic role for miR-155 through the induction of apoptosis and myeloid differentiation in the AML cell line OCI-AML3 (11), whereas several groups have reported miR-155 as an oncogene in leukemia (14,16-19) and therefore a potential therapeutic target (16). Based on the published data, it appears that the function of miR-155 is context-dependent. Narayan *et al.* recently proposed a novel dose-dependent role for miR-155 in the regulation of AML, which might partially explain the observed discrepancy (20). Considering that miR-155 has hundreds of predicted targets, determining its regulated downstream pathways is highly context-dependent and thus challenging. Of note, although miR-155 is highly expressed in murine hematopoietic stem and progenitor cells (HSPCs), miR-155$^{-/-}$ mice did not show impaired myeloid differentiation or any perturbations in the HSPC compartment (21).

Wallace *et al.* now uncovered the downstream mechanism of miR-155 in FLT3-ITD AML (22). Mice homozygous for FLT3-ITD (FLT3-ITD$^{+/+}$) develop a myeloproliferative disease (MPD) defined by a myeloid-specific cell expansion, however, the mutation itself does not drive leukemic transformation. Interestingly, a comparable phenotype was observed through engineered overexpression of miR-155 in HSPCs. Through crossing homozygous FLT3-ITD (FLT3-ITD$^{+/+}$) mice with miR-155 knock out (miR-155$^{-/-}$) mice generating FLT3-ITD$^{+/+}$/miR-155$^{-/-}$ mice, Wallace *et al.* showed that deletion of miR-155 in FLT3-ITD homozygous mice weakens the MPD phenotype, suggesting a functional relationship. More importantly, this direct genetic approach resolved previous controversies about the functional relationship of miR-155 and FLT3-ITD in AML cells.

FLT3-ITD$^{+/+}$/miR-155$^{-/-}$ mice had a decreased myeloid progenitor compartment compared to FLT3-ITD$^{+/+}$ mice as well as reduction in proliferation of LSK and myeloid progenitor cell populations, indicating a role for miR-155 in promoting myeloid progenitor expansion in the pre-malignant context of FLT3-ITD mediated MPD. Using an RNA sequencing approach of sorted LSK cells, the authors identified the interferon pathway as highly enriched in FLT3-ITD$^{+/+}$/miR-155$^{-/-}$ *vs.* FLT3 ITD$^{+/+}$ LSK cells. This was further confirmed by Western blot analysis of STAT1, a master regulator of interferon responses, which showed highly increased STAT1 protein levels in FLT3-ITD$^{+/+}$/miR-155$^{-/-}$ cells. Based on this finding, the authors concluded that miR-155 promotes proliferation of myeloid progenitor cells by reducing the anti-proliferative effects of interferon signaling in FLT3-ITD$^{+/+}$ leukemia. By mining their hypothesis with the TCGA-LAML dataset, the authors confirmed that IFN-α and IFN-γ were significantly downregulated in FLT3-ITD AML compared with FLT3-WT AML. Subsequent genetic depletion through CRISPR/Cas9 of miR-155 in human AML cell lines showed elevated Interferon signaling and STAT1 levels. To identify the relevant targets of miR-155 in the context of FLT3-ITD mutated AML, the authors showed the upregulation of established miR-155 targets including Ship1, Pu.1 and Cebpb in FLT3-ITD$^{+/+}$/miR-155$^{-/-}$ compared to FLT3-ITD$^{+/+}$ mice and further confirmed the expression levels of these targets in the human TCGA-LAML dataset. CEBPb is a known interferon regulator whereas Ship1 is an inhibitor of AKT signaling, demonstrating that miR-155 works through several targets to modulate multiple signaling pathways and responses in FLT3-ITD mutated AML. Finally, Wallace *et al.* translated their findings to primary human FLT3-ITD mutated AML cells, in which they could demonstrate reduced survival and increased apoptosis after treatment with a miR-155 inhibitor *in vitro*.

The findings of Wallace *et al.* highlight the relevance of miR-155 in FLT3-ITD driven AMLs and open a new path to the possibility that the depletion or inhibition of miR-155 may provide a therapeutic angle. While much has been learned about miR-155 biology, there are still many unanswered questions that add complexity to its role in AML and it is yet to be determined if inhibition of this miRNA *in vivo* is ready for prime time and will eventually lead to a better outcome for AML patients.

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Footnote

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