Abstract: P456

Title: OVERCOMING AGGRESSIVE EMT-ASSOCIATED PHENOTYPES IN ACUTE MYELOID LEUKEMIA CELLS WITH KMT2A-MLLT3 REARRANGEMENTS THROUGH THE MODULATION OF MICRORNA-204

Abstract Type: Poster Presentation

Topic: Acute myeloid leukemia - Biology & translational research

Background:

Genetic sequencing of acute myeloid leukemia (AML) with *KMT2A* rearrangements (*KMT2A*r) has facilitated the creation of MENIN inhibitors, marking the first targeted therapy for this patient subgroup. These inhibitors attenuate the aberrant upregulation of the stem cell genes *HOXA9* and *MEIS1*, which promote aggressive disease in affected patients. Recently, downstream targets of this complex with defined roles in the epithelial to mesenchymal transition (EMT) in solid tumors have been shown to promote stemness, invasiveness, and chemoresistance in AML. Identifying novel regulators of these effectors is crucial to overcoming refractory disease and relapse, as we continue to unravel the vast intratumoral heterogeneity within patients. Given the critical role of *MEIS1* in *KMT2A*r leukemic stem cells, and prior evidence linking increased *miR-204* expression to its downregulation and improved outcomes in AML patients, our objective was to investigate the tumor suppressor role of *miR-204* in *KMT2A-MLLT3* AML.

Aims:

Through the modulation of *miR-204* expression, this study aims to elucidate gene targets and pathways driving aggressive phenotypes and contributing to refractory disease and relapse in *KMT2A-MLLT3* rearranged AML.

Methods:

We used lentiviral overexpression of *miR-204* in human and murine models of *KMT2A-MLLT3* AML to investigate its anti-leukemic effects through a variety of *in vivo* and *in vitro* assays.

Results:

To resolve the reported *miR-204/MEIS1* axis, we used transplantable murine models driven by *KMT2A-MLLT3* or by the co-overexpression of its downstream effectors *Hoxa9* and *Meis1,* with predicted *miR-204* binding sites removed. The overexpression of *miR-204* significantly prolonged survival in *KMT2A-MLLT3* mice from 51 days to 74 days, as well as in *Hoxa9/Meis1* mice from 50 days to 75 days despite the truncated *Meis1* 3'UTR, suggesting an alternative mechanism of *miR-204*. Cell morphology and immunophenotype assessed *ex vivo* were consistent with increased myeloid differentiation through *miR-204*, and bone marrow from *KMT2A-MLLT3* mice was sent for RNA-sequencing and proteomics. Notably, analysis did not show a downregulation of *Hoxa9* or *Meis1* but instead revealed the repression of downstream genes including regulators of EMT and chemotaxis with well-established clinical significance in t(9;11) AML. We used our murine model and a human CD34+ cord blood model of *KMT2A-MLLT3* to assess aggressive phenotypes shown to be driven by EMT-associated genes *in vitro*. Overexpression of *miR-204* caused* a significant reduction in colony forming capacity (CFC) in our human AML model yet did not alter lineage outputs of healthy CD34+ cord blood. Similarly, overexpression of *miR-204* in our murine *KMT2A-MLLT3* cells significantly reduced CFC output and increased sensitivity to cytarabine. As we confirmed the low expression of *miR-204* in AML patient samples, we sought to restore the expression at the endogenous locus in *KMT2A-MLLT3* AML cells. The repression of *miR-204* has been correlated with the hypermethylation of its host gene promoter (*TRPM3*) in an array of other cancers, so we took advantage of the approved AML hypomethylating agent 5-azacytidine. We observed that repeated low-dose treatment increased expression of *miR-204* in both human and murine models of *KMT2A-MLLT3*.

Summary/Conclusion:

Our results define an anti-leukemic role of *miR-204* in *KMT2A-MLLT3* AML, where its increased expression represses EMT-associated genes downstream of the HOXA9/MEIS1 complex that are driving aggressive disease phenotypes. Targets revealed in this study hold therapeutic promise in novel treatment strategies for patients with t(9;11) AML.

Keywords: KMT2A, Meis1, AML, HOXa9