Title Expression and Function of Long non-coding RNAs in Acute Myeloid Leukemia

AUTHORS: Ramiro Garzon, MD

Purpose: Long noncoding RNAs (lncRNAs) are transcripts longer than 200 nucleotides located within the intergenic stretches or overlapping antisense transcripts of protein coding genes. While lncRNAs contribute to epigenetic gene regulation, metastasis and prognosis in solid tumors, their role in acute myeloid leukemia (AML) has not been deeply characterized. Here, we investigate the expression and function of lncRNAs in AML.

Experimental Design: To determine whether lncRNAs are associated with clinical features, recurrent mutations and outcome in older patients (aged ≥60 years) and younger (<60 years) with cytogenetically normal (CN) AML, we evaluated lncRNA expression in 219 older and 375 younger untreated CN-AML cases using RNA sequencing. Functional experiments were carried out using cell proliferation, apoptosis and colony formation assays in primary AML cells and cell lines after knock-down using custom LNA gapmers. Comparative Proteomic analysis was performed by applying a modified version of the RNA antisense purification technique.

Results: We found that a small subset of lncRNAs is correlated strongly with outcome. Furthermore distinctive lncRNA expression patterns were identified for the most frequent recurrent mutations in CN-AML. Among the top up-regulated lncRNAs in NPM1 mutated (NPM1mut) CN-AML cases, the lncRNA HOXB-AS3 was identified. We confirmed the up-regulation of HOXB-AS3 in a large cohort of young NPM1mut CN-AML patients. We showed that HOXB-AS3 knockdown lead to a decrease blast proliferation and colony formation in AML cell lines and primary AML patients in vitro. Silencing HOXB-AS3 in vivo resulted in an increased survival of treated patient derived xenograft mice with respect to controls. Comparative proteomics identified several RNA binding protein partners of HOXB-AS3, such as EBP1, which is associated with ribosomal biogenesis. Further experiments indicated that HOXB-AS3 binds to EBP1 and regulates ribosomal biogenesis in AML by affecting the interactions between EBP1 and NPM1 complex.

Conclusion: We found that a small subset of lncRNAs is correlated strongly with treatment response and survival in both younger and older CN-AML patients. Furthermore distinctive lncRNA expression patterns were identified for recurrent mutations in CN-AML. Furthermore, our preliminary data supports that HOXB-AS3 plays an important role in NPM1mut AML, and blocking HOXB-AS3 may be a viable therapeutic target in NPM1mut AML.