SOHO ANNUAL MEETING 2024

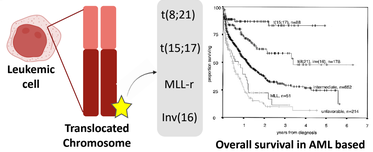
Exploring Potential Molecular Dependency of t (8;21)(q22;q22.1) Positive Acute Myeloid Leukemia on the Non-Coding RNA HOTAIRM1-miR222 Axis

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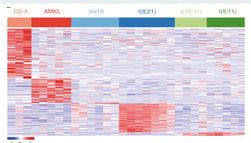
INTRODUCTION

① Recurrent translocations impact patient survival



Overall survival in AML based on cytogenetic subgroups

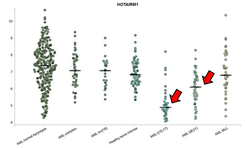
② Distinct non-coding RNA signature in AML with chromosomal translocations



③ HOTAIRM1 (HOXA Transcript Antisense RNA, Myeloid-Specific 1)

- First long non-coding RNA found to **regulate myeloid lineage differentiation** *(Zhang et al., 2009)*.
- Highest expression in mature granulocytes "fingerprint lncRNA of granulopoiesis" *(Schwarzer et al., 2017)*.

④ HOTAIRM1 Expression in AML



AIM

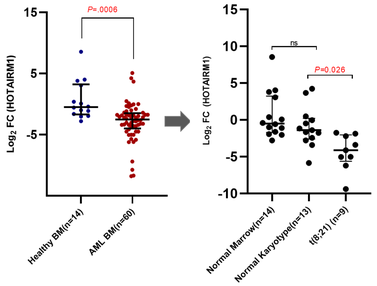
To study significance of dysregulated lncRNA HOTAIRM1 in AML

METHODS

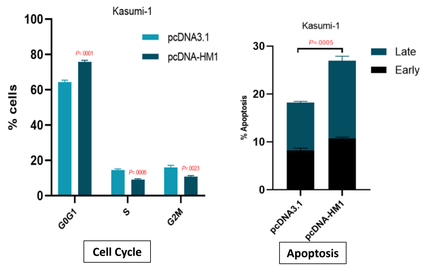
A	**Gene Expression Analysis**	
	Expression of HOTAIRM1	Quantitative PCR (qPCR) was performed on RNA extracted bone Marrow(BM) of patients with AML(n=60) & BM from patients with normal marrow(n=14). For *in vitro* experiments, qPCR was performed on Kasumi-1 cell line in triplicates.
	Expression of microRNAs & apoptotic pathway mRNA	For *in vitro* experiments, qPCR was performed on Kasumi-1 cell lines in triplicates.
B	**Functional Analysis of HOTAIRM1 Pathway**	
1	**HOTAIRM1 Expression Modulation**	HOTAIRM1 full length sequence was cloned in an over-expression vector (pcDNA3.1) & transient transfection was carried out.
2.	**In-vitro assays for cell cycle and apoptosis**	The assay was performed on a flow cytometer by staining Kasumi-1 cells with 7-AAD (cell cycle) and Annexin and 7-AAD (apoptosis).
3.	**Protein Expression Analysis**	Immunofluorescence and Flow Cytometry
C	**Statistical Analysis**	
1	Comparison between groups	Independent t-test (two groups), Kruskal Wallis Test (more than two groups)
2	Survival Analysis	Kaplan Meier survival analysis

RESULTS

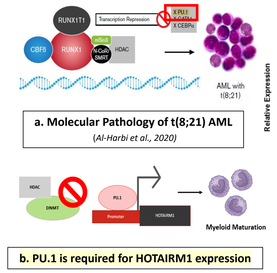
① HOTAIRM1 is downregulated in t(8;21) AML

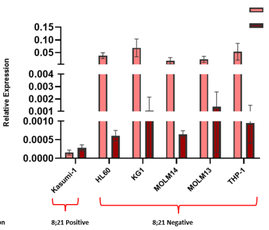


② HOTAIRM1 inhibits cell cycle and induces apoptosis in t(8;21) positive Kasumi-1 Cell Line

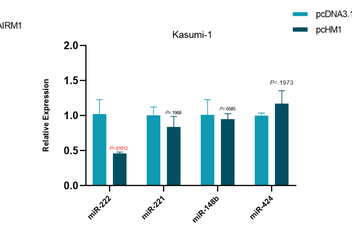


③ Protein complex involving RUNX1/RUNX1T1 oncoprotein represses myeloid transcription factor PU.1

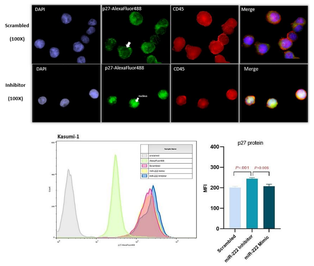




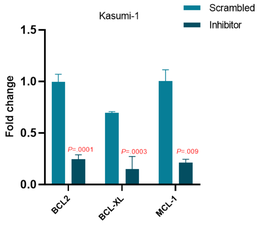
④ HOTAIRM1 suppresses oncogenic miR-222



⑤ Inhibiting miR-222 leads to upregulation of cell cycle inhibitor p27/CDKN1B in Kasumi-1

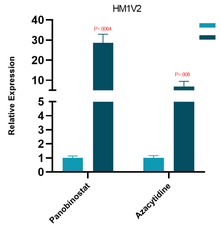


⑥ Inhibition of miR-222 reduces expression of anti-apoptotic genes BCL2, BCL-xL and MCL-1

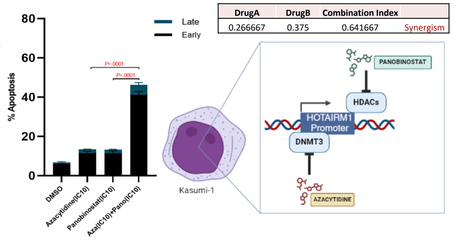


⑦ Treatment of Kasumi-1 cells with Azacytidine(HMA) & Panobinostat(HDACi)

a. Rescues HOTAIRM1 expression

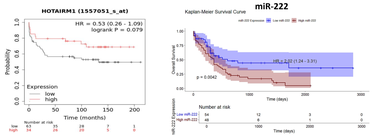


b. Induces Kasumi-1 apoptosis



DrugA	DrugB	Combination Index	
0.266667	0.375	0.641667	Synergism

⑧ Clinical Significance of HOTAIRM1 & miR-222



Datasets	Gene	Hazard Ratio (CI)	P-value
GSE 1159, 12417, 37642, 6891, 8970	HOTAIRM1 High	0.53(0.26-1.09)	0.079
TCGA L-AML Dataset	miR-222 High	**2.02(1.24-3.31)**	**0.0042**

CONCLUSIONS

1. LncRNA HOTAIRM1 has a tumor suppressive role and is downregulated in t(8;21) AML.
2. The oncogenic effect of high miR-222 expression is abrogated by inducing HOTAIRM1. Drugs Panobinostat and Azacytidine increase HOTAIRM1 expression and induce apoptosis in a synergistic manner in t(8;21) positive Kasumi-1 cell line.
3. High expression of miR-222 is significantly associated with poor overall survival in patients with AML (HR=2.02, P-value=0.0042).

ACKNOWLEDGEMENTS

Funding:

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- X. Zhang *et al.*, "A myelopoiesis-associated regulatory intergenic noncoding RNA transcript within the human HOXA cluster," *Blood*, vol. 113, no. 11, pp. 2526–2534, Mar. 2009, doi: 10.1182/blood-2008-06-162164.
- A. Schwarzer *et al.*, "The non-coding RNA landscape of human hematopoiesis and leukemia," *Nat. Commun.*, vol. 8, no. 1, p. 218, Aug. 2017, doi: 10.1038/s41467-017-00212-4.

CONTACT INFORMATION

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