Poster Number: 4

MetroHealth Medical Center

RESEARCH DAY 2023

Abstract Submission Form

Poster Title: **The interplay of RNA N^6-methyladenine machinery and long noncoding RNA fuels tyrosine kinase inhibitor resistance through PI3K signaling in leukemia**

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Category: **Cancer Biology**

Background: Acquired resistance to tyrosine kinase inhibitors (TKI), such as nilotinib used to treat leukemia, leads to disease recurrence and is frequently driven by non-genetic factors. Long non-coding RNAs (lncRNAs) have been linked to drug resistance, yet the regulatory mechanisms and the molecular functions of lncRNA dysregulations in resistant cancers remains poorly understood.

Experimental Design: We established resistant leukemia models by chronic exposure of leukemia cells to the physiologically attainable concentrations of nilotinib. We performed transcriptomic and epitransciptomic profiling in long-term nilotinib-deprived leukemia cells to identify pathways required for the development and maintenance of drug resistance. We carried out Western blot, immunoprecipitation, qPCR, and dotblot to characterize changes in proteins, DNA and RNAs of the lead candidates and elucidate the engaged mechanisms. We employed phenotypic assays such as CCK-8, flow cytometry and clonogenic assays to assess the effects of ectopic expression, genetic silencing and drug sensitivity. Finally, the therapeutic effects of PI3K-targeting drug were tested in leukemic mouse models.

Results: We show that many differentially expressed lncRNAs enrich N^6-methyladenosine (m6A), and more lncRNAs tend to have higher m6A content in leukemia cells resistant to nilotinib. The top-ranked lncRNAs, including PROX1-AS1, SENCR and LN892, are highly elevated in nilotinib resistant cell lines, TKI non-responding patients and leukemia patients with blast crisis when compared with their respective counterparts. Patients with higher levels of lncRNAs (PROX1-AS1, SENCR, LN892) survive shorter than those with lower expression. Knockdown of these lncRNAs impairs resistant cell growth and renders resistant cells sensitive to nilotinib-induced cell death. Mechanistically, lncRNA upregulation is attributed to FTO-dependent m6A hypomethylation that stabilizes lncRNA transcripts, and empowers resistant cell growth through the activated PI3K signaling. Treatment with PI3K inhibitor Alpelisib sensitizes nilotinib resistant cells to nilotinib-induced cell death and reduces leukemia burden in mice.

Conclusion: These findings establish a surprising non-genetic paradigm for the FTO-m6A-lncRNA nexus in fueling leukemia drug resistance, and warrant clinical exploration of lncRNAs as well as PI3K inhibitor Alpelisib as single or combinational reagents in treating refractory/relapse leukemia patients.