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Acute Leukemias

TARGETING THE PA2G4-NPM1 AXIS TRIGGERS NUCLEOLAR STRESS IN NPM1C AML

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Introduction: : NPM1 mutation, the most common genetic alteration in acute myeloid leukemia (AML), remains a therapeutic limitation. Although preclinical strategies targeting NPM1 showed promise, clinical translation has been limited. Developing compounds that selectively target NPM1-mutated (NPM1c) AML cells, especially in high-risk molecular contexts, endures as a major translational challenge.

Methods: : We identified PA2G4 interactors in AML cells by PA2G4 pulldown followed by nLC-MS/MS and validated PA2G4-NPM1 interaction by co-immunoprecipitation (co-IP). We functionalized a PA2G4 inhibitor, WS6, with a biotin tag on the terminal piperazine ring for anti-biotin co-IP studies. We assessed WS6 effects in NPM1c models using *in vitro* functional assays, analyzed transcriptomic changes by RNA sequencing, and tested *in vivo* efficacy in an NPM1c patient-derived xenograft (PDX) model.

Results: : Our group previously demonstrated that the RNA-binding protein PA2G4 functions as a bystander of Myc signaling in 3q26 AML. Given the role of Myc in high-risk AML, we subsequently extended our investigation of PA2G4 to broader AML subtypes. From the mass spectrometry (MS) analysis of PA2G4 interactors (n=167, $P<0.05$), we isolated NPM1. Low-throughput validation confirmed that PA2G4 binds NPM1 in AML NPM1c cells and that shRNA-mediated *PA2G4* knockdown abrogates this interaction. To confirm WS6 specificity we generated a WS6-biotinylated derivative and from tag pulldown confirmed the predicted binding supporting our study.

NPM1c cell lines and primary samples were highly sensitive to WS6 (IC50 0.008-1.8µM). Notably, NPM1c cells were more sensitive than NPM1 wild-type (WT) cells ($P=0.001$), while other drugs showed no difference. Furthermore, WS6 reduced NPM1c levels more than WT isoforms, supporting the hypothesis that NPM1c sensitizes AML cells to PA2G4 inhibition. To further explore how WS6 exerts its effects in NPM1c cells, we treated IMSM2 cells for 24 h and performed RNA sequencing, identifying 1071 upregulated and 1269 downregulated genes ($adj.P<0.05$), enriched for ribosome biogenesis ($adj.P=2.64\times10^{-18}$) and E2F targets ($adj.P=1.99\times10^{-48}$), consistent with nucleolar stress induction suggesting a potential mechanism of action for WS6. As such, we analyzed NPM1 localization after WS6 treatment showing nucleolar disaggregation with NPM1 WT relocating to the nucleoplasm and NPM1c accumulating near the nuclear membrane. As expected from the proposed mechanism, NPM1 redistribution promoted p53 stabilization. Finally, we evaluated the anti-leukemic effect of WS6 in an NPM1c PDX model treated with 25 mg/kg for 15 days. WS6 reduced $CD45^+$ bone marrow cells ($P<0.05$), quantitative *NPM1c* transcripts (NPM1c/Control=0.00086) and decreased Ki-67, a marker of cell proliferation ($P<0.0001$).

Conclusions: : Our results highlight PA2G4 as a potential therapeutic target in NPM1c AML, particularly in cases where NPM1c appears to lose its association with a favorable prognosis.