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S122 ROCK INHIBITORS TARGET SRSF2 LEUKEMIA BY DISRUPTING CELL MITOSIS AND NUCLEAR MORPHOLOGY

Topic: 03. Acute myeloid leukemia - Biology & Translational Research

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Background: Spliceosome machinery mutations are common early mutations in myeloid malignancies, however effective targeted therapies against them are still lacking in clinical settings.

Aims: Exploring safe and efficient methods to target hematopoietic cells carrying *SRSF2* mutations might be a powerful tool not just for leukemia prevention and treatment, but also to understand the mechanisms behind *SRSF2* mutations.

Methods:

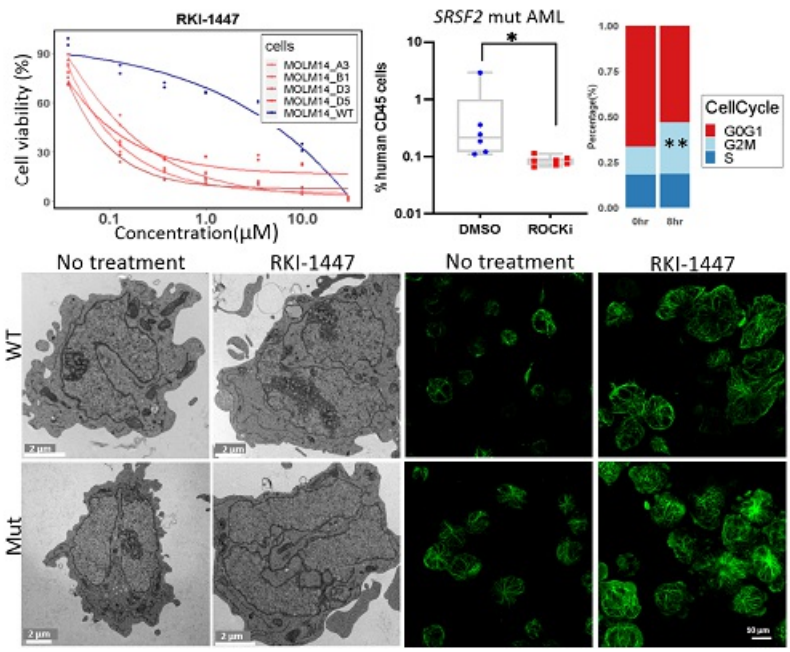
We generated five *SRSF2* Mut (P95H) isogenic cell lines with CRISPR/CAS9 system and performed high throughput drug screen with 3988 compounds in a single dose. After we narrowed down our targets, dose response assay was performed on four different ROCK inhibitors (ROCKi). The leading ROCKi compound was validated *in vivo* with *SRSF2* Mut AML xenograft models. Next, we aimed at understanding why ROCKi target *SRSF2* Mut cells and used proteomics, gene expression and imaging of the cytoskeletal system to study the effect of ROCKi on Mut and WT cells.

Results:

In the current study, we used an in vitro high-throughput drug screen among four different isogenic cell lines and identified ROCK inhibitors (ROCKi) as selective inhibitors of *SRSF2* Mut in MOLM14 and AML2 cells. To study the efficacy of RKI-1447 on human samples, we conducted six AML patient-derived xenograft (PDX) experiments, with *SRSF2* Mut samples. In four out of the six primary AML samples, RKI-1447 significantly reduced engraftment compared to the untreated group and inhibited the engraftment of both leukemic blasts and pre leukemic-HSPCs (preL-HSPCS). RKI-1447 was not toxic to mice nor human cells. ROCKi induced mitotic catastrophe (G2M arrest and multipolar spindles) through their apparent effects on microtubules and nuclear organization, and leading to cell death. Confocal imaging and transmission electron microscopy (TEM) data revealed that *SRSF2* mutations induce deep nuclear indentation and segmentation, which is reminiscent of the nuclear shape of Pelger–Huët anomaly (PHA). The nuclear volume and area were significantly higher in *SRSF2* Mut compared with WT MOLM14 cells regardless of ROCKi. To investigate why *SRSF2* Mut cause the structural changes, we looked into the cytoplasmic intrusions that segment the nuclei, and the structures that connect the lobes of the nuclei by TEM. This examination revealed enrichment of microtubule bundles inside the nuclear indentations. More importantly, these microtubules were

located at the tip of the cytoplasmic intrusions suggesting that they take an active role in the nuclear segmentation process. After exposure to RKI-1447, the cytoskeletal and nuclear morphological abnormalities of *SRSF2* Mut are augmented to a level which are incompatible with cell survival.

Image:



Summary/Conclusion:

We believe it is the first report describing in high resolution the changes in the nucleus after *SRSF2* mutations are introduced. The mechanisms we identified are most probably relevant to other SMMs and to the dysplastic phenotype observed in MDS and AML. Accordingly, our findings have wide implications as ROCKi might be even more useful than describe here. Altogether, we provide data from many directions that ROCKi should be tested in clinical trials.

Abstract Book Citations: Authors, Title, HemaSphere, 2022;6:(S3):pages. The individual abstract DOIs can be found at https://journals.lww.com/hemasphere/pages/default.aspx.