(PS1960) RUXOLITINIB COMBINED WITH HOMOHARRINGTONINE SYNERGISTICALLY TARGETS CDK2 IN RUXOLITINIB-RESISTANT MYELOFIBROSIS AND SECONDARY ACUTE MYELOID LEUKEMIA

Topic: 15. Myeloproliferative neoplasms - Biology & translational research

Rumeng Li[1], Qingmei Han[1], Tian Zeng[1], Xiudi Yang[1], Jian Huang*[1]

[1]*The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China;*

Background

Myeloproliferative neoplasms (MPNs) frequently develop secondary resistance and progress to secondary acute myeloid leukemia (sAML), which demonstrates poor response to JAK inhibitors and confers extremely unfavorable prognosis.

Aims

This study aims to investigate the efficacy and mechanism of ruxolitinib combined with homoharringtonine (HHT) in the treatment of JAK-mutant drug-resistant MPNs and sAML.

Methods

We performed single-cell RNA sequencing in ruxolitinib-resistant and -sensitive MPN patients and established drug-resistant JAK2-mutant cell lines. CDK2 expression was examined in clinical samples and cell lines. Drug combination effects were evaluated by CellTiter-Lumi assay and validated in patient samples. Apoptosis and cell cycle were analyzed by flow cytometry. Key pathways were identified by RNA-seq and proteomics and validated by RT-qPCR and Western blot. A xenograft model was used to assess tumor burden, survival, and toxicity.

Results

Single-cell sequencing revealed a significantly higher proportion of hematopoietic stem cells (HSCs) in resistant patients, whereas B cells and T cells were more abundant in sensitive patients. Additionally, CDK2 expression was upregulated in resistant patients and exhibited high expression in established resistant cell lines. Further studies demonstrated that CDK2 was universally overexpressed in MPNs, with expression levels further elevated in patients with leukemic transformation; notably, high CDK2 expression was closely associated with increased sensitivity to HHT. Based on these findings, we employed a ruxolitinib combined with homoharringtonine regimen. Cell viability assays indicated that combination therapy significantly inhibited cell proliferation, induced apoptosis, and produced stronger G1-phase arrest compared with monotherapy. RNA-seq, proteomics, and GSEA analyses showed that combination treatment specifically suppressed E2F, PI3K-AKT, mTORC1, c-Myc, and JAK-STAT signaling pathways; meanwhile, both single-cell sequencing and resistant cell RNA-seq data confirmed significant activation of the PI3K/AKT pathway in resistant cells. Western blot and qPCR verified that the two-drug combination exerted synergistic effects through the AKT/mTOR/MYC/CDK2/RB axis to promote cell apoptosis. In NSG mouse models, combination therapy significantly reduced leukemia burden, markedly prolonged mouse survival, and did not increase toxicity.

Summary/Conclusion

This study demonstrates that CDK2 is highly expressed in MPNs, with expression levels further elevated in patients with leukemic transformation; high CDK2 expression is closely associated with increased sensitivity to HHT. Ruxolitinib combined with homoharringtonine synergistically induces cell cycle arrest, inhibits pro-survival signaling, and activates apoptosis through the AKT/mTOR/MYC/CDK2/Cyclin A2/pRB axis, providing a novel therapeutic strategy for MPN patients with leukemic transformation and high CDK2 expression, with promising clinical translational potential.

This research was supported by the National Natural Science Foundation of China (No. 82570191) and the Zhejiang Provincial Health Highlevel Innovative Talent Project (2022-2026).* Correspondence to:Jian Huang, email: househuang@zju.edu.cn