Romidepsin targets leukemia stem cell to overcome drug resistance in acute myeloid leukemia

HYPOTHESIS:
Leukemia stem cells (LSC) are related to chemoresistant in acute myeloid leukemia. LSC markers CD123 and CD47 may serve as biomarkers for chemoresistant AML cells. Therapies targeting CD123+CD47+ population can potentially help parents overcome drug-resistant and relapse.

BACKGROUND/AIMS:
Acute myeloid leukemia (AML) is the most common acute leukemia in adults. Cytarabine (Ara-C) has been the main chemotherapy drug for the treatment since the 1960s. Although over 70% of the patients can achieve remission after the first round of treatment, around 50% to 70% of first remission patients relapse within 3 years, and most relapse with the development of drug-resistance. Recent studies suggest that AML relapse is caused by the survival of drug resistant leukemia stem cell (LSC) population. In this study, we generated a chemoresistant cell line as a model to study the mechanism of chemoresistant, and also its relationship with LSC.

METHODS:
We generated a drug-resistant cell line from an AML cell line, OCI-AML2, by gradually increasing the Ara-C concentration in culture media. Flow cytometry was used to identify cells with different cell surface markers. We also performed RNA-seq on cells with different levels of drug resistance. Mouse xenograft model was used to test the drug effect in vivo.

RESULTS & CONCLUSIONS
To better understand the mechanism of drug resistance and LSC, we collected various of human AML cell lines to exam correlations of their IC50 of Ara-C and the percentage of individual LSC markers in these cell lines. We found that commonly accepted LSC marker, such as CD34, CD44, CD13, CD117, CD96 and CD45RA, are not correlated with cell drug sensitivities. Interestingly, CD47 and CD123 are positively correlated with chemo resistance. In order to rule out heterogeneous genetic background effect, we generated a drug-resistant cell line from an AML cell line, OCI-AML2, by gradually increasing the Ara-C concentration in culture media. During the selection, the percentage of cells with cancer stem cells makers is gradually increased. After the resistant cell lines are established, we found the resistant cell lines grow slower comparing with parent cell line. RNA-seq data shows, genes that differentially expressed in resistant lines are enriched in cell cycle and DNA repair pathways. However, the genes that related to stemness, such as OCT4, SOX2, MYC and KLF4, didn't change much in drug-resistant cells compare with the sensitive strand. Therefore, chemoresistant cells may represent cells in a slow growing, high LSC marker population, but not bare all characteristics of LSC.

We further investigate the role of epigenetic factors in regulating the survival of chemoresistant leukemia cells. Our data shows epigenetic drugs that targeting histone deacetylases, bromodomain proteins and PRC1 complex are effective in inducing apoptosis and cell death in chemoresistant cells, therefore providing a potential second line chemotherapy drug for chemoresistant patients. Furthermore, we found histone deacetylase inhibitor Romidepsin have synergetic effect when combining with Ara-C. Moreover, a single treatment of Ara-C enriched slow growing, high LSC marker cell population, however Romidepsin treatment is more tend to target this population. Furthermore, the combination of Romidepsin and Ara-C could target both fast and slow growing cell and lower leukemia stem cell marker positive population, achieving better treatment effect. Xenograft in NSG mouse model also confirmed this result.

In summary, our study sheds light on a new mechanism of drug resistance in leukemia, and provide a rationale to develop and test epigenetic-targeted therapies in leukemia, especially in drug-resistant relapse patients.