ELSEVIER

Blood 142 (2023) 1368

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Linking DNA Damage Response to Ferroptosis for Leukemia Stem Cell Eradication

Bilal Malik, PhD[1], *Nunki Hassan, PhD*[2], *Jenny Y. Wang, PhD*[3]

[1] Kolling institue, University of Sydney, St Leonards, Sydney, Australia
[2] Kolling Institute, University of Sydeny, St Leonards, Sydney, Australia
[3] Kolling Institute, University of Sydney, St Leonards Sydney, AUS

Relapse in acute myeloid leukemia (AML) is associated with an unfavorable prognosis, primarily due to the poor response to conventional chemotherapy, which has shown disappointing overall outcomes over the past 30 years. The main culprits behind leukemogenesis and therapy resistance are leukemia stem cells (LSCs), characterized by their unique features of self-renewal and evasion from cell death. In our recent work, we have identified a critical self-renewal pathway, RSPO3-LGR4/p-PKAc, which exhibits aberrant activation in relapsed or refractory AML (Salik et al. *Cancer Cell*, 2020). Blocking this pathway holds the potential to eradicate resistant LSCs and treat relapsed AML effectively.

In this study, our data directly link the self-renewal pathway to ferroptosis resistance through activation of antioxidant defense, which confers protection on AML LSCs against detrimental effects of oxidative stress. We also found that aberrant activation of RSPO3-LGR4/p-PKAc suppressed the DNA damage response (DDR) pathway consequently leading to genomic instability, DNA repair defects, increased stemness and AML aggressiveness. Our whole-genome sequencing and knockout mouse studies showed that deletion of the key DDR gene caused substantial mutations on multiple pathways including DNA damage repair (e.g., Slf1) and self-renewal (e.g., Hox cluster genes), contributing to a more aggressive and therapy-resistant phenotype in AML initiation and progression. Single cell multi-omics sequencing and CRISPR/Cas9 knockout mouse models uncovered essential antioxidant enzymes as downstream targets of DDR pathway inhibition in AML patient-derived LSCs. These antioxidant enzymes induced by DDR pathway inhibition had protective effects against oxidative attack due to the ability to decompose reactive oxygen species (ROS) and detoxify excess iron. ShRNA-mediated stable knockdown of antioxidant enzymes impaired LSCs by increasing accumulation of iron and ROS and sensitized LSCs to ferroptosis (an iron-dependent and ROS-reliant cell death). Our analysis of AML dataset from TCGA (The Cancer Genome Atlas, *N Engl J Med* 2013) displayed a positive correlation of high expression of antioxidant genes with poor prognosis in a cohort of 172 AML patients ($p<0.019$), indicating the clinical importance of antioxidant dysregulation in cancer.

Collectively, we report here a functional relationship of DDR pathway inhibition and antioxidant defense mechanism that is regulated by RSPO3-LGR4/p-PKAc self-renewal pathway and contributes to ferroptosis resistance in AML LSCs. Targeting essential pathway components enables LSCs to become sensitive to oxidative damage and ferroptosis and perturbs leukemia progression, presenting a promising new therapeutic approach for relapsed or refractory AML.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-182752