Mitochondrial Respiration Regulates GPX4 Inhibition-Induced Ferroptosis in Acute Myeloid Leukemia

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Background
Ferroptosis, a form of non-apoptotic cell death regulated by iron-dependent lipid peroxidation, has drawn extensive attention as potential anti-cancer strategy. However, it remains to be explored in hematologic malignancies. We here investigate the molecular mechanisms of ferroptosis in acute myeloid leukemia (AML) and its therapeutic potential with co-targeting of mitochondrial respiration.

Hypothesis
Ferroptosis pathway is a therapeutic vulnerability in AML
• Oxidative stress and iron overload in AML cells
• Induction of cell death that bypasses apoptosis resistance

Materials and Methods
Public datasets; 'shyDepMap', GEP1A, CRISPR screening for NALM6 cells treated with ClpP agonist
Cells; Parental AML cell lines, OCI-AML3-shGPX4, MOLM-13-shGPX4, Kasumi-1-shTP53, HL60-Rho0
Primary AML patient samples
Reagents; GPX4 inhibitor; ML210, ClpP agonist; ONC201, lipophilic antioxidants; Liproxstatin-1 (Lip1) and a-Tocopherol (aToc), iron chelator; deferoxamine (DFX), mitochondrial antioxidants; MitoQ and MitoHQ2
Flow cytometry; AnnexinV/DAPI cell death assay, C11, BODIPY581/591 and MitoPerOx lipid peroxidation assays, MitoSOX red mitochondrial superoxide indicator
Western blot
Transmission electron microscopy

Results
GPX4 is a potential therapeutic target in AML with prognostic relevance

GPX4 inhibition induces ferroptosis in AML cells

GPX4 knockdown by doxycycline-inducible shRNA induces lipid peroxidation followed by cell death in AML cells

GPX4 inhibition by ML210 induces lipid peroxidation and cell death in AML cell lines. The pharmacologic effects are blocked by lipophilic antioxidants and the iron chelator DFO.

Mitochondrial respiration protects AML cells from ferroptosis

Respiration-deficient HL60-Rho0 cells are more sensitive to GPX4 inhibition

Mitochondrial respiration protects AML cells from ferroptosis

Mitochondrial respiration regulates GPX4 inhibition-induced ferroptosis in AML cell lines. The pharmacologic effects are blocked by lipophilic antioxidants and the iron chelator DFO.

ClpP-mediated degradation of mitochondrial respiratory complex proteins sensitizes AML cells to ferroptosis

Combination of ClpP hyperactivation (ONC201 or dox-induced CLPP-Y118A hyperactivated mutant) and GPX4 inhibition (ML210 or dox-induced knockdown) in AML cell lines and primary AML progenitor (CD34⁺/CD38⁻) cells

Summary
• GPX4 inhibition induces ferroptosis in AML cells
• Mitochondrial respiration protects AML cells from GPX4-mediated ferroptosis
• ClpP-mediated degradation of mitochondrial respiratory complex proteins and GPX4 inhibition synergistically exerts anti-leukemia effects
• Studies are in progress to determine the molecular mechanisms and the in vivo efficacy of the combination

Acknowledgement

Reference