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

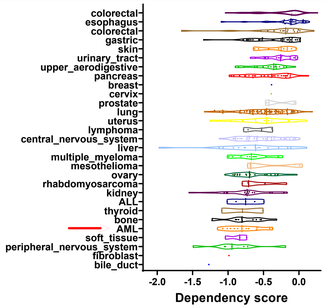
Public datasets: shinyDepMap[1], GEPIA[2], CRISPR screening for NALM6 cells treated with ClpP agonists[3]
Cells; Parental AML cell lines, OCI AML3-shGPX4, MOLM13-TP53mut[4], Kasumi1-shTP53, HL60-Rho0[5], OCI-AML3-CLPP-Y118A[6], Primary AML patient samples
Reagents; GPX4 inhibitor; ML210, ClpP agonist; ONC201, lipophilic antioxidants; Liproxstatin-1 (Lip1) and a-Tocopherol (aToc), iron chelator; deferoxamine (DFX), mitochondrial antioxidants; MitoQ and MitoQH2
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

References

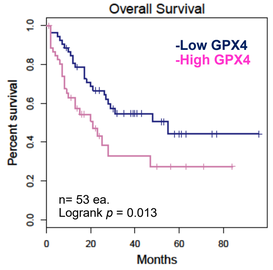
1. Shimada, K., et al. eLife 2021;10:e57116
2. Tang, Z., et al. Nucleic Acids Research 2017;45, 98–102.
3. Jacques, S., et al. Genetics 2020;214:1103–1120.
4. Boettcher, S., et al. Science 2019;365(6453):599–604.
5. Pelicano, H., et al. Journal of Biological Chemistry 2003;278(39):37832–37839.
6. Ishizawa, J., et al. Cancer Cell 2019;35(5):721-737.e9.

Results

GPX4 is a potential therapeutic target in AML with prognostic relevance



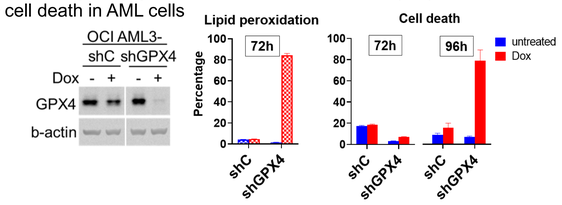
AML cell lines are one of the cell lines highly dependent (low dependency score) on GPX4 among those on DepMap (shinyDepMap)



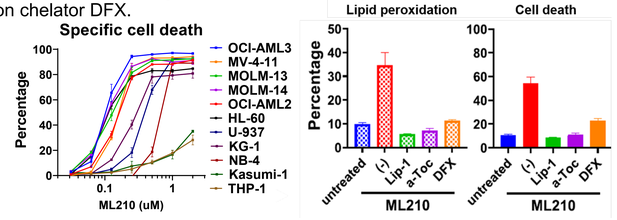
High GPX4 mRNA expression associates with shorter survival in AML patients (TCGA)

GPX4 inhibition induces ferroptosis in AML cells

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

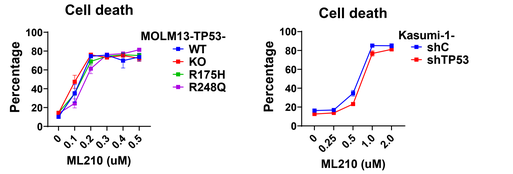


GPX4 inhibition by ML210 induces lipid peroxidation and cell death in AML cell lines. The pharmacologic effects are blocked by lipophilic antioxidants and the iron chelator DFX.

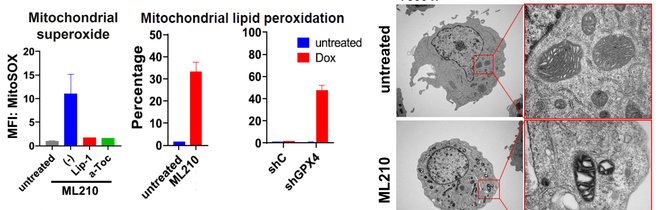


Anti-leukemia effect of GPX4 inhibition is independent of TP53 mutational status

TP53 modulations in MOLM13 (CRISPR-mediated gene editing) or Kasumi-1 (knockdown of mutant p53) do not change the ML210 sensitivity



GPX4 inhibition induces lipid-associated oxidative stress and ultrastructural alterations in mitochondria

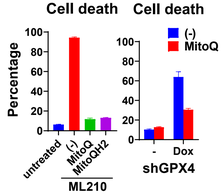


GPX4 inhibition induces

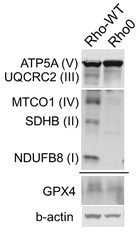
- mitochondrial superoxide blocked by lipophilic antioxidants
- mitochondrial lipid peroxidation

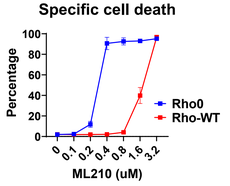
Small mitochondria with condensed membrane in ML210-treated cells

Mitochondrial respiration protects AML cells from ferroptosis



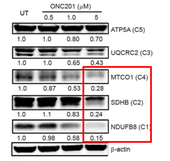
MitoQ/QH2 block the anti-leukemia effects of GPX4 inhibition

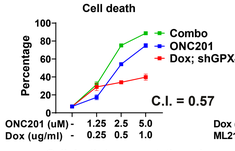


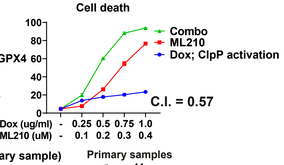


Respiration-deficient HL60-Rho0 cells are more sensitive to GPX4 inhibition

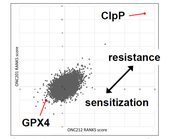
ClpP-mediated degradation of mitochondrial respiratory complex proteins[6] sensitizes AML cells to ferroptosis



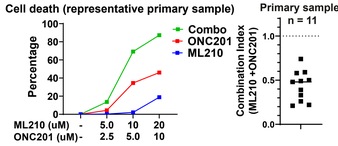




ONC201 degrade respiratory complex proteins (published[6])



GPX4 was one of the top sensitization hits on CRISPR screening for ClpP hyperactivation



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**

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