Synergistic Induction of Ferroptosis in Acute Myeloid Leukemia by Modulating Intracellular Lipidomic Remodeling

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Introduction

Acute myeloid leukemia (AML) therapy remains highly challenging. Acquired resistance to apoptosis-based treatment underscores an urgent need for alternative strategies. **Ferroptosis, a non-apoptotic form of cell death**, has emerged as a promising approach to potentially overcome cancer therapy resistance in context-dependent manners. However, the mechanisms and therapeutic implications of ferroptosis in AML are still not well understood. Ferroptosis is triggered by the oxidation of various lipids in membrane phospholipids (PLs), mostly **polyunsaturated fatty acid (PUFA)** tails, and the accumulation of lipid peroxides. PUFA dynamics are tightly regulated in cells through coordinated processes of uptake, storage, distribution, and metabolism. **Lipid droplets (LDs)** are intracellular regulatory organelles for lipid dynamics.

Aim

To investigate the strategy for enhancing ferroptosis for AML therapy by modulating intracellular PUFA and LD biogenesis.

Methods

AML cell lines OCI-AML3, MOLM13, and Doxycycline-inducible GPX4 knockdown AML cells were used in this study.
For ferroptosis induction, cells were treated with ML210 or GPX4i to inhibit GPX4 or treated with Doxycycline to knock down GPX4.
Cells were treated with A922500 (DGAT1 inhibitor), docosahexaenoic acid (DHA, PUFA), linoleic acid (PUFA), or oleic acid (MUFA) as indicated.
AnnexinV & DAPI staining was used for cell death assay by flow cytometry.
Lipid droplet formation in cells was analyzed by BODIPY 493/503 staining.
Mass-spectrometry-based lipidomics was performed for lipid profiling.

Results

1. Ferroptosis is enhanced by PUFA but abrogated by MUFA

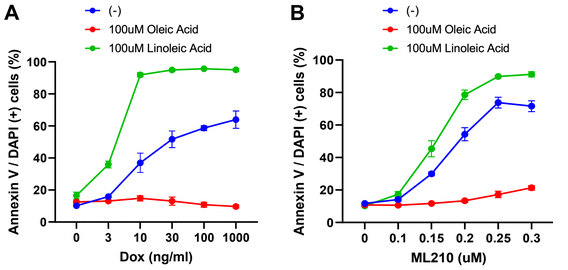


Fig.1. PUFA (Linoleic acid) enhances while MUFA (Oleic acid) eliminates ferroptosis. Cell death in OCI-AML3 cells treated with Linoleic acid or Oleic acid during ferroptosis induced by GPX4 knockdown (A) or GPX4 inhibitor ML210 (B). Doxycycline (Dox) was used to induce shGPX4 expression. Ferroptotic cells was measured using flow cytometry with Annexin V and DAPI staining 48h after treatments.

2. GPX4 inhibition decreases intracellular PUFA-phosphatidylethanolamine (PE)

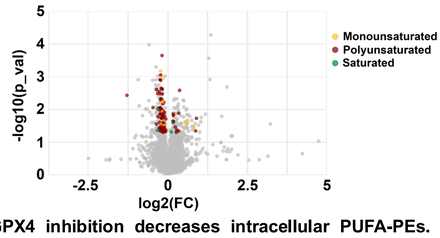


Fig.2. GPX4 inhibition decreases intracellular PUFA-PEs. OCI-AML3 cells were treated with 250nM ML210 for 8h and followed by mass-spectrometry-based lipidomics analysis.

3. GPX4 inhibition increases PUFAs in triglycerides (TGs)

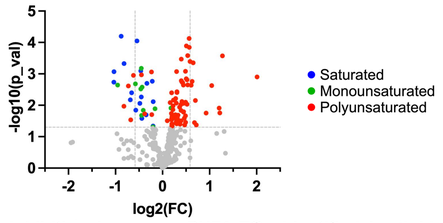


Fig.3. GPX4 inhibition increases PUFA-TGs identified by lipidomics. OCI-AML3 cells were treated with 250nM ML210 for 48h and followed by mass-spectrometry-based lipidomics analysis.

Given TGs are mainly stored in lipid droplets (LDs), we hypothesized that cells adapt to ferroptosis by inducing sequestration of pro-ferroptotic PUFAs into LDs, thereby, buffering ferroptotic stress, and that exogenous PUFA supplementation and inhibition of LD formation would enhance ferroptosis.

4. Unsaturated fatty acids or GPX4 inhibition increases lipid droplet formation

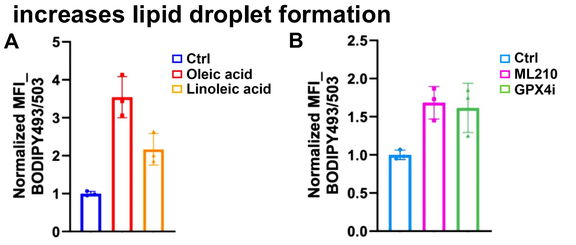


Fig.4. LD formation upon the treatments of unsaturated fatty acids or GPX4 inhibitors. (A) LD formation in OCI-AML3 cells when treated with PUFA (Linoleic acid, 100μM) or MUFA (Oleic acid, 100μM). (B) LD formation in OCI-AML3 cells when treated with ML210 (0.15μM) or an unpublished GPX4 inhibitor GPX4i (7.5nM). LD formation in the cells after 24h treatment was determined by flow cytometry with BODIPY 493/503 staining.

5. DGAT1 knockout reduces lipid droplet formation and enhances ferroptosis in AML cells

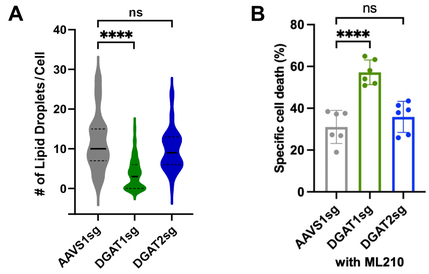


Fig. 5. LD formation in DGAT1/2-deficient AML cells and ferroptosis induced by GPX4 inhibitor ML210. (A) DGAT1-KO significantly reduced LD formation, but DGAT2-KO did not. LD number in MOLM13 cells was counted on the images with BODIPY 493/503 staining. (B) DGAT1-KO significantly enhanced ferroptosis induced by ML210, but DGAT2-KO did not.

6. DGAT1 inhibition decreases lipid droplet formation

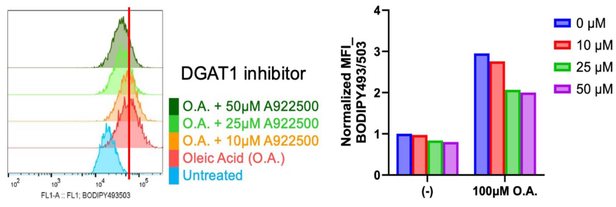


Fig.6. Inhibition of DGAT1 decreases LD formation induced by oleic acid. (A) LD formation in OCI-AML3 cells when treated with oleic acid and a DGAT1 inhibitor A922500. LD formation in cells after 24h treatment was determined by BODIPY 493/503 staining measured by flow cytometry.

7. Synergistic cell death induced by combination of GPX4i, DGAT1i, and exogenous PUFA

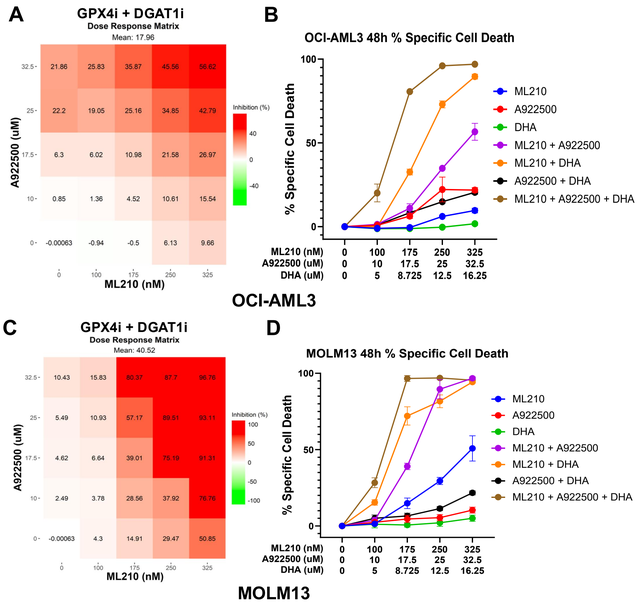
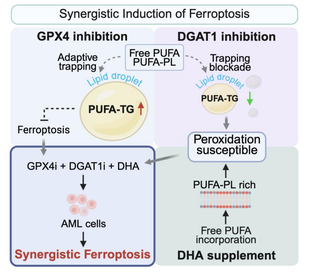


Fig.7. Synergistic effects of GPX4i, DGAT1i and PUFA treatment in ferroptosis in AML Cells. (A,C) Dose response matrix depicting the percentage of cell death upon various concentrations of ML210 and A922500 treatments in OCI-AML3 (A) and MOLM13 (C) cells. (B,D) Cell death after treatments with ML210, A922500, or DHA for 48 hours.

Summary

- Ferroptosis in AML cells is enhanced by exogenous PUFA
- GPX4 inhibition-induced ferroptosis induces intracellular lipid remodeling, increases PUFAs in TGs
- Exogenous lipids or GPX4 inhibition-induced ferroptosis increases LD formation in AML cells
- DGAT1 inhibition/KO decreases LD formation and enhances ferroptosis in AML cells
- The combination of GPX4i, DGAT1i and PUFA treatment exerts synergistic cell death in AML



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