RXR agonist IRX4204 induces ferroptosis in multiple myeloma via HMOX1/GPX4 axis and enhances lenalidomide efficacy

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INTRODUCTION

Multiple myeloma (MM) remains incurable despite current therapies, highlighting the need for new strategies to overcome drug resistance. Retinoid X receptors (RXRs) are ligand-activated transcription factors and serve as obligate heterodimerization partners for multiple nuclear receptors. RXR signaling controls lipid metabolism, oxidative stress response, and immune regulation. We have previously shown that pharmacologic activation of RXR enhanced the anti-myeloma efficacy of lenalidomide. IRX4204, a third-generation RXR agonist, demonstrated safety and anti-cancer activities in preclinical and phase I and II clinical studies.

AIM

This study aimed to investigate whether pharmacologic activation of the retinoid X receptor (RXR) by the selective agonist IRX4204 enhances lenalidomide efficacy in vitro and in vivo in preclinical myeloma models. We further sought to determine the effects and underlying mechanism of IRX4204 on myeloma cell ferroptosis, a form of iron-dependent cell death characterized by the accumulation of lipid peroxides

METHOD

- **In vitro studies:** MM1.R and U266 cells were treated with IRX4204, ferroptosis inducer RSL3, or inhibitor Ferrostatin-1. Cell viability, lipid peroxidation (BODIPY-C11), and intracellular Fe^{2+} (BioTracker dye) were analyzed by MTT and flow cytometry.
- **Mechanistic assays:** Western blotting, immunofluorescence, ChIP-qPCR, and HMOX1 promoter-luciferase assays evaluated ferroptosis-related proteins and PPARα/RXRα binding. HMOX1 knockout cells were generated using CRISPR/Cas9.
- **In vivo model:** SCID mice bearing MM1.R xenografts received IRX4204, lenalidomide, or combination therapy to assess tumor growth, survival, and ferroptosis biomarkers.

RESULTS

1. IRX4204 sensitizes multiple myeloma cells to ferroptosis.

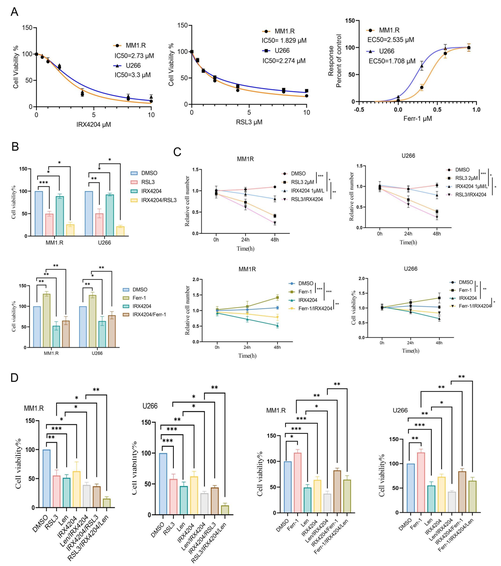

Fig1 (**A**) Dose–response curves of MM1.R and U266 cells treated with IRX4204, RSL3, or Ferr-1. (**B**) The MM1.R and U266 cell lines were treated with DMSO, RSL3 (2 μM), IRX4204 (3 μM), or the combination for 48 h. Cell viability was assessed by the MTT assay. (**C**) Cell number measured at 0, 24, and 48 h showing enhanced growth inhibition with combination treatment. (**D**) Cell viability after lenalidomide (Len, 10 μM), IRX4204, RSL3, or combined treatment for 48h. All experiments performed in triplicate; *p<0.05, **p<0.01, ***p<0.001.

2 IRX4204 reduces ferroptosis-protective proteins, thereby elevating ferroptosis stress.

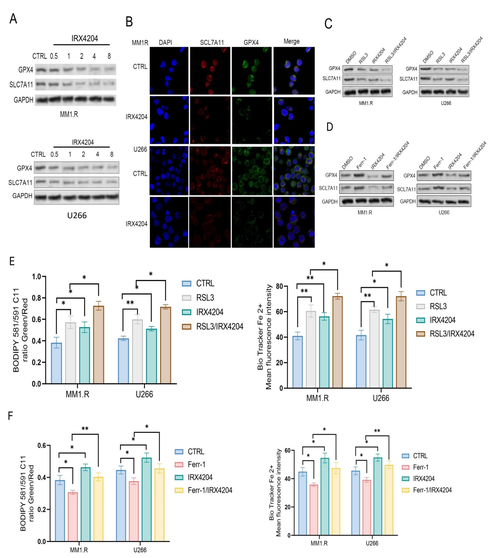

Fig2 (**A**) IRX4204 dose-dependently reduced GPX4 and SLC7A11. (**B**) Immunofluorescence confirmed decreased GPX4/SLC7A11. (**C–D**) Ferroptosis inducer (RSL3) enhanced, while inhibitor (Fer-1) rescued IRX4204-mediated GPX4 suppression. (**E**) IRX4204 increased lipid peroxidation (BODIPY C11). (**F**) Intracellular Fe^{2+} levels increased (Far-Red Fe^{2+} dye). *p<0.05, **p<0.01, **p<0.001.*

3. HMOX1 is a ferroptosis-associated gene linked to RXR signaling and clinical outcomes.

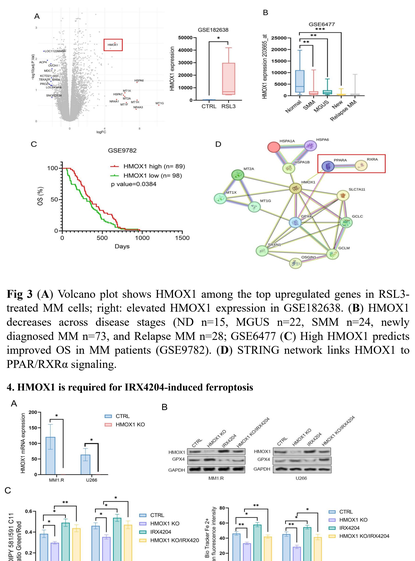

Fig 3 (**A**) Volcano plot shows HMOX1 among the top upregulated genes in RSL3-treated MM cells; right: elevated HMOX1 expression in GSE182638. (**B**) HMOX1 decreases across disease stages (ND n=15, MGUS n=22, SMM n=24, newly diagnosed MM n=73, and Relapse MM n=28; GSE6477 (**C**) High HMOX1 predicts improved OS in MM patients (GSE9782). (**D**) STRING network links HMOX1 to PPAR/RXRα signaling.

4. HMOX1 is required for IRX4204-induced ferroptosis

Fig4 (**A**) qPCR confirms efficient HMOX1 knockout. (**B**) Western blot shows that HMOX1 KO mitigates IRX4204-mediated GPX4 reduction in MM1.R and U266 cells. (**C**) HMOX1 KO reduces IRX4204-induced lipid peroxidation (BODIPY C11) and Fe^{2+} accumulation (Far-Red Fe^{2+} dye). *p<0.05, **p<0.01, **p<0.001.*

5. IRX4204 transcriptionally activates HMOX1 through PPAR/RXR binding.

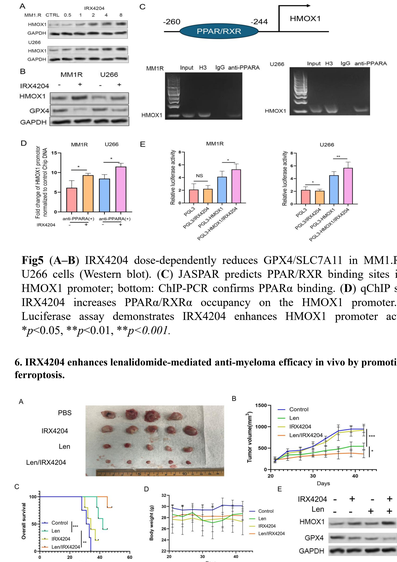

Fig5 (**A–B**) IRX4204 dose-dependently reduces GPX4/SLC7A11 in MM1.R and U266 cells (Western blot). (**C**) JASPAR predicts PPAR/RXR binding sites in the HMOX1 promoter; bottom: ChIP-PCR confirms PPARα binding. (**D**) qChIP shows IRX4204 increases PPARα/RXRα occupancy on the HMOX1 promoter. (**E**) Luciferase assay demonstrates IRX4204 enhances HMOX1 promoter activity. *p<0.05, **p<0.01, **p<0.001.*

6. IRX4204 enhances lenalidomide-mediated anti-myeloma efficacy in vivo by promoting ferroptosis.

Fig6 (**A**) Representative images of SCID mice bearing subcutaneous MM tumors. (**B**) IRX4204 potentiates lenalidomide-mediated tumor growth inhibition. (**C**) Kaplan–Meier survival analysis shows prolonged survival with the combination. (**D**) Body weight monitored every 3 days; no significant toxicity observed. (**E**) Tumor lysates analyzed by immunoblotting for indicated markers.

CONCLUSIONS

- **Mechanistic insight:** IRX4204 activates RXR signaling and induces ferroptosis in multiple myeloma by transcriptionally upregulating HMOX1 and suppressing GPX4, uncovering a distinct RXR–HMOX1–GPX4 regulatory pathway.
- **Experimental validation:** Loss-of-function studies confirmed that HMOX1 is indispensable for IRX4204-induced ferroptosis, as its knockout abolished lipid peroxidation and iron accumulation.
- **Therapeutic implication:** In vivo, IRX4204 synergized with lenalidomide to inhibit tumor growth and prolong survival without detectable toxicity, supporting RXR-mediated ferroptosis as a promising therapeutic approach for relapsed or refractory multiple myeloma.

REFERENCES

- Jabbar S, Mathews P, Wang X, Sundaramoorthy P, Chu E, Piryani SO, Ding S, Shen X, Doan PL, Kang Y: Thioredoxin-1 regulates self-renewal and differentiation of murine hematopoietic stem cells through p53 tumor suppressor. Exp Hematol Oncol 2022, 11(1):83.
- Zheng C, Zhang B, Li Y, Liu K, Wei W, Liang S, Guo H, Ma K, Liu Y, Wang J et al: Donafenib and GSK-J4 Synergistically Induce Ferroptosis in Liver Cancer by Upregulating HMOX1 Expression. Adv Sci (Weinh) 2023, 10(22):e2206798.
- Wu J, Wang X, Zhang M, Mathews P, Kang Y: RXR Agonists Enhance Lenalidomide Anti-Myeloma Activity and T Cell Functions while Retaining Glucose-Lowering Effect. Cells 2023, 12(15).

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