

Article

Celastrol induces ferroptosis in multiple myeloma associated with suppression of the squalene synthase-mediated lipid defense axis

Huizi Ye¹, Bin Peng^{1,*}

1 Shanghai Health Commission Key Lab of Artificial Intelligence (AI)-Based Management of Inflammation and Chronic Diseases, Gongli Hospital of Shanghai Pudong New Area, Shanghai University of Medicine & Health Sciences, Shanghai, China

*** Corresponding author(s):**

Bin Peng, pb01312@glhospital.com

Received: 30 August 2026

Accepted: 18 September 2026

Published: 30 September 2026

© 2026 The Author(s). AI Med published by AI Press.

This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0).

Abstract: Multiple myeloma (MM) remains incurable due to pervasive drug resistance, motivating exploration of non-apoptotic cell death pathways. Squalene synthase (SQS, encoded by FDFT1), the first committed enzyme in sterol biosynthesis within the mevalonate (MVA) pathway, has been linked to ferroptosis defense, but its role in MM is poorly defined. This study aimed to investigate whether SQS modulates celastrol-induced ferroptosis in MM. Four MM cell lines (RPMI-8226, U266, KMS-11, SKO-007) were treated with celastrol. Cell viability, intracellular iron, reactive oxygen species (ROS), malondialdehyde (MDA), and glutathione (GSH) were measured. Ferroptosis inhibitors (ferrostatin-1, deferoxamine mesylate) and lentiviral SQS overexpression were used to assess functional dependence. Bulk RNA-seq data from the MMRF-COMMPASS cohort (n = 764 primary CD138⁺ plasma cell samples) were analyzed for FDFT1-ferroptosis correlations and pathway activity using GSVA and GSEA. Celastrol reduced viability (24-h IC₅₀: 2.41-4.44 μmol/L) with iron accumulation, ROS elevation, MDA increase, and GSH depletion in all four cell lines. Ferrostatin-1 and deferoxamine partially rescued viability and restored SQS, GPX4, SLC7A11, and FSP1 protein levels. SQS overexpression partially protected RPMI-8226 cells and mitigated iron/MDA accumulation while preserving GSH. In MMRF-COMMPASS, FDFT1-high tumors showed significantly elevated ferroptosis defense pathway activity (GSVA P_{adj} = 1.37 × 10⁻⁶; GSEA NES = 2.13, P_{adj} = 2.68 × 10⁻³), although FDFT1 was not an independent prognostic marker (univariate HR = 1.064, P = 0.441; multivariate HR = 1.059, P = 0.482). SQS is a functional ferroptosis defense node in MM. Celastrol-induced ferroptosis is associated with suppression of the MVA-SQS axis, supported by pharmacological, genetic, and clinical transcriptomic evidence. These findings provide a preclinical rationale for targeting SQS-mediated metabolic vulnerability in MM.

Keywords: celastrol; squalene synthase; FDFT1; multiple myeloma; ferroptosis; mevalonate pathway

Multiple myeloma (MM) is a plasma-cell malignancy characterized by substantial biological heterogeneity and recurrent treatment resistance [1,2]. Although proteasome inhibitors, immunomodulatory drugs, monoclonal antibodies, and stem-cell transplantation have improved outcomes, relapse remains nearly universal, and the disease is considered incurable [3,4]. This persistent therapeutic failure underscores the urgent need to identify non-apoptotic cell death pathways and metabolic dependencies that can be exploited to overcome resistance in refractory MM clones [5,6].

Ferroptosis is an iron-dependent form of regulated cell death driven by catastrophic lipid peroxidation, distinct from apoptosis, necroptosis, and autophagy [7,8]. The canonical defense axis comprises the cystine/glutamate antiporter SLC7A11, glutathione (GSH), and glutathione peroxidase 4

(GPX4), which collectively suppress phospholipid peroxidation [9,10]. A parallel glutathione-independent system centered on ferroptosis suppressor protein 1 (FSP1) and coenzyme Q10 (CoQ10) provides additional protection at the plasma membrane [11,12], while mitochondrial dihydroorotate dehydrogenase contributes a third layer of defense [13]. In MM specifically, ferroptosis inducers have shown promising preclinical activity, and iron metabolism is increasingly recognized as a determinant of bortezomib response [14-17]. Furthermore, the bone marrow microenvironment can modulate MM cell ferroptosis sensitivity through stromal-cell-driven lanosterol biosynthesis [18].

The mevalonate (MVA) pathway is the central biosynthetic route for sterols, including cholesterol, CoQ10,

and isoprenoid intermediates [19,20]. Squalene synthase (SQS, encoded by FDFT1) catalyzes the first committed step in sterol biosynthesis, converting two molecules of farnesyl pyrophosphate to squalene [21,22]. MVA-derived metabolites, particularly CoQ10 and squalene itself, have been implicated in ferroptosis defense [23-25]. Notably, squalene accumulation protects certain lymphomas and solid tumors from oxidative cell death [23], whereas the consequences of SQS suppression in hematological malignancies remain poorly defined.

Celastrol is a pentacyclic triterpenoid, itself an MVA pathway product, isolated from *Tripterygium wilfordii* [26], with broad anti-inflammatory and antitumor activities [27,28]. In MM, celastrol inhibits proliferation and induces apoptosis through NF- κ B and proteasome inhibition [29,30]. Recent studies have linked celastrol to ferroptosis in multiple cancer types [31-33], but whether ferroptosis contributes to its anti-MM activity and which metabolic nodes are involved remain unknown.

In the present study, we investigated whether SQS modulates celastrol-induced ferroptosis in MM. We assessed ferroptosis-associated phenotypes in four human MM cell lines, evaluated the functional contribution of SQS through pharmacological rescue and genetic overexpression, and interrogated the clinical transcriptomic context of FDFT1 in the MMRF-COMMPASS cohort (n = 764). Our findings identify SQS as a functional ferroptosis defense node whose suppression is associated with celastrol-induced cell death in MM.

2. Materials and Methods

2.1. Cell Culture and Reagents

RPMI-8226 (Chinese Academy of Sciences, Shanghai, China), U266, KMS-11, and SKO-007 (gifts from Prof. Jian Hou, Changzheng Hospital, Shanghai, China) were maintained in RPMI-1640 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified 5% CO₂ incubator. All cell lines were confirmed mycoplasma-negative. Celastrol (purity $\geq$ 98%, Sigma-Aldrich, St. Louis, MO, USA; catalog no. C0869; molecular formula C₂₉H₃₈O₄, molecular weight 450.61 g/mol) was dissolved in dimethyl sulfoxide (DMSO) as a 50 mmol/L stock; the final DMSO concentration in culture medium did not exceed 0.1% (v/v). Ferrostatin-1 (Fer-1; SML0583) and deferoxamine mesylate (DFOM; D9533) were purchased from Sigma-Aldrich. Antibodies used were: SQS (ab236666, Abcam, Cambridge, UK), GPX4

(52455, Cell Signaling Technology, Danvers, MA, USA), SLC7A11 (HA721868, HuaAn Biotechnology, Hangzhou, China), FSP1 (24972S, Proteintech, Rosemont, IL, USA), SREBP2 (ab30682, Abcam), and GAPDH (D11005, Sangon Biotech, Shanghai, China).

2.2. Cell Viability Assay

Cells were seeded in 96-well plates at a density of $1-2 \times 10^4$ cells per well and treated with celastrol at concentrations of 0-10 μ mol/L for 24, 48, or 72 h. For rescue experiments, Fer-1 (5 μ mol/L) or DFOM (10 μ mol/L) was added 1 h before celastrol treatment. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. Absorbance at 450 nm was measured using a BioTek microplate reader (Agilent, Santa Clara, CA, USA). Half-maximal inhibitory concentration (IC₅₀) values were calculated by non-linear regression using GraphPad Prism 10.0 (GraphPad Software, San Diego, CA, USA).

2.3. Biochemical Assays and ROS Detection

After 24 h of celastrol treatment, intracellular iron concentration was measured using a colorimetric iron assay kit (NJCBIO, Nanjing, China). Malondialdehyde (MDA) levels were quantified using the thiobarbituric acid reactive substances (TBARS) assay kit (Beyotime Biotechnology, Shanghai, China). Reduced glutathione (GSH) was measured using a GSH assay kit (Beyotime Biotechnology). All measurements were normalized to total protein concentration determined by the bicinchoninic acid (BCA) method. Intracellular reactive oxygen species (ROS) were detected using 10 μ mol/L 2',7'-dichlorofluorescein diacetate (DCFH-DA; Beyotime Biotechnology). Cells were incubated with DCFH-DA for 30 min at 37 °C, washed twice with phosphate-buffered saline, and imaged using an Olympus IX73 fluorescence microscope (Olympus, Tokyo, Japan). Mean fluorescence intensity was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA). All assays were performed in triplicate and repeated at least three times.

2.4. Western Blotting

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (Beyotime Biotechnology) containing protease and phosphatase inhibitor cocktails. Protein concentration was determined by the BCA assay. Equal amounts of protein (20-30 μ g per lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to

polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% non-fat milk for 1 h at room temperature and incubated with primary antibodies overnight at 4 °C. After washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology) for 1 h at room temperature. Signals were developed using enhanced chemiluminescence reagent (Thermo Fisher Scientific, Waltham, MA, USA) and imaged on a ChemiDoc MP system (Bio-Rad, Hercules, CA, USA). Densitometric analysis was performed using ImageJ with GAPDH as the loading control.

2.5. Lentiviral SQS Overexpression

A lentiviral vector expressing human FDFT1 (SQS) with a GFP reporter and puromycin resistance cassette was constructed by Obio Technology (Shanghai, China). RPMI-8226 cells were transduced at a predetermined multiplicity of infection in the presence of 6-8 µg/mL polybrene. Stable transductants were selected with 2 µg/mL puromycin for 2 weeks, after which GFP-positive cells exceeded 90% as confirmed by fluorescence microscopy. SQS overexpression was validated by western blotting. An empty vector-transduced cell line served as the negative control.

2.6. Bioinformatic Analysis of MMRF-COMMPASS Data

Bulk RNA-seq data and corresponding clinical information from the MMRF-COMMPASS study were retrieved via TCGAblinks [34]. Primary bone marrow CD138⁺ plasma cell samples (n = 764) were selected for analysis. Gene-level raw counts were normalized using the variance-stabilizing transformation implemented in DESeq2 [35]. Pearson correlation coefficients between FDFT1 expression and 24 core ferroptosis-related genes were calculated, with Benjamini-Hochberg (BH) false discovery rate (FDR) correction applied for multiple testing. Survival analysis was performed using Kaplan-Meier estimation and Cox proportional hazards regression via the survival R package [36], with multivariate models adjusted for age, sex, and International Staging System (ISS) stage. Pathway activity was estimated using gene set variation analysis (GSVA) with single-sample gene set enrichment analysis (ssGSEA) [37], utilizing custom ferroptosis-related gene sets and Hallmark/KEGG gene sets from the Molecular Signatures Database (msigdb) [38]. Differential expression between FDFT1-high and FDFT1-low groups (defined by median split) was analyzed using limma [39], and gene set enrichment analysis (GSEA) was conducted using fgsea [40]. A two-sided

$P < 0.05$ or $FDR < 0.05$ was considered statistically significant.

2.7. Statistical Analysis

Wet-lab experiments were performed at least three independent times. Data are presented as mean ± standard deviation (SD). Comparisons between two groups were performed using the two-tailed Student's t-test. Multiple group comparisons were analyzed by one-way or two-way analysis of variance (ANOVA) followed by Dunnett's post hoc test, as appropriate. All statistical analyses for experimental data were conducted using GraphPad Prism 10.0. For bioinformatic analyses, statistical significance was defined as $P < 0.05$ or $FDR < 0.05$ after BH correction.

3. Results

3.1. Celastrol Reduces MM Cell Viability and Induces Ferroptosis-Associated Biochemical Changes

Celastrol reduced the viability of RPMI-8226, U266, KMS-11, and SKO-007 cells in a concentration- and time-dependent manner (Figure 1a). The 24-h IC_{50} values were 2.41, 2.66, 3.30, and 4.44 µmol/L, respectively, with RPMI-8226 showing the greatest sensitivity. After 24 h of treatment, celastrol significantly increased intracellular iron and MDA levels, decreased GSH content (Figure 1b-d), and elevated DCFH-DA fluorescence, indicating increased ROS production (Figure 1e), in all four cell lines. These changes are consistent with oxidative stress and a ferroptosis-associated phenotype.

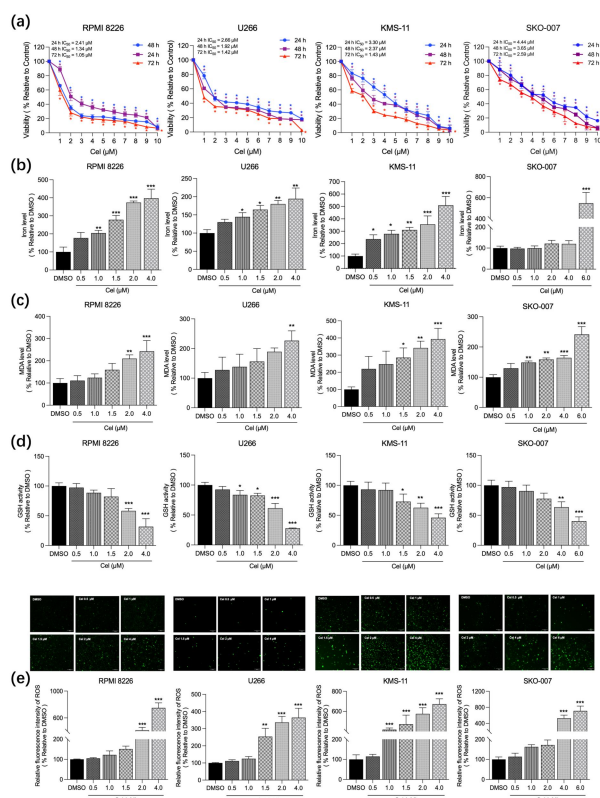


Figure 1. Celastrol reduces MM cell viability and induces ferroptosis-associated biochemical changes. (a) Viability of RPMI-8226, U266, KMS-11, and SKO-007 cells after treatment with celastrol for 24, 48, or 72 h, measured by CCK-8 assay. (b-d) Intracellular iron, malondialdehyde (MDA), and glutathione (GSH) levels after 24 h of celastrol treatment. (e) Representative DCFH-DA fluorescence images and quantification of mean fluorescence intensity after 24 h. Data represent mean $\pm$ SD (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 vs. DMSO control.

3.2. Celastrol Suppresses SQS and Ferroptosis-Defense Proteins; Inhibitors Rescue the Phenotype

Celastrol treatment for 24 h suppressed SQS protein abundance in a concentration-dependent manner in all four MM cell lines (Figure 2a). The ferroptosis-defense proteins GPX4, SLC7A11, and FSP1, as well as the MVA pathway master regulator SREBP2, were also reduced. To confirm ferroptosis dependence, Fer-1 (a lipid peroxidation inhibitor) and DFOM (an iron chelator) were employed. Both inhibitors partially attenuated celastrol-induced loss of viability (Supplementary Figure S1), reduced celastrol-associated ROS elevation, iron accumulation, and MDA production, and partially restored GSH levels in RPMI-8226 cells (Supplementary Figure S2). Western blot analysis demonstrated that both Fer-1 and DFOM significantly reversed celastrol-mediated suppression of SQS, GPX4,

SLC7A11, and FSP1 in RPMI-8226 cells (Figure 2b). The incomplete rescue indicates that ferroptosis contributes to celastrol-induced cytotoxicity but may not be the sole cell death mechanism involved.

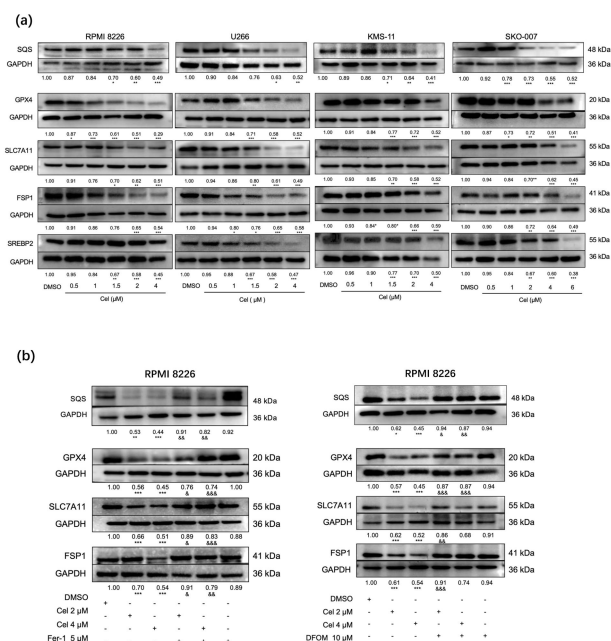


Figure 2. Celastrol suppresses SQS and ferroptosis-defense proteins; Fer-1 and DFOM rescue. (a) Representative western blots and densitometric quantification of SQS, GPX4, SLC7A11, FSP1, and SREBP2 upon celastrol treatment for 24 h in RPMI-8226, U266, KMS-11, and SKO-007 cells. (b) Representative western blots for SQS, GPX4, SLC7A11, and FSP1 in RPMI-8226 cells pre-treated with Fer-1 (5 μ mol/L) or DFOM (10 μ mol/L) before celastrol exposure. Numbers below each blot indicate densitometric quantification normalized to GAPDH. Data are presented as mean $\pm$ SD (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 vs. DMSO control; &P < 0.05, &&P < 0.01, &&&P < 0.001 vs. respective celastrol-treated group.

3.3. SQS Overexpression Partially Protects RPMI-8226 Cells from Celastrol

To provide genetic evidence for the functional role of SQS, stable SQS-overexpressing RPMI-8226 cells were generated. SQS protein abundance was increased by approximately 1.38-fold relative to the empty vector control (Figure 3a, b). Compared with vector control cells, SQS-overexpressing cells showed significantly greater viability after celastrol exposure (Figure 3c), reduced intracellular iron and MDA levels, and partial preservation of GSH (Figure 3d-f). These results provide genetic evidence that SQS expression levels modulate celastrol sensitivity and ferroptosis-associated biochemical changes in MM cells.

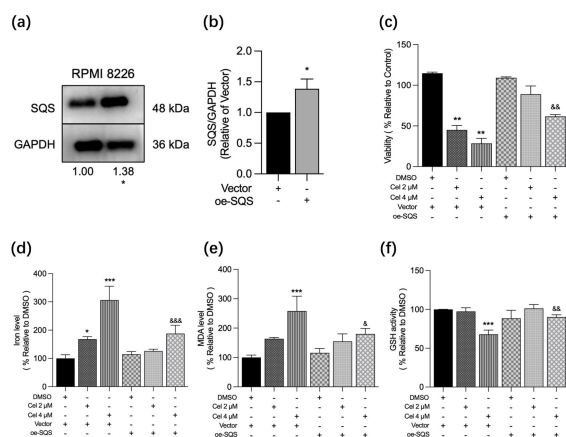


Figure 3. SQS overexpression partially protects RPMI-8226 cells from celastrol-induced cytotoxicity. (a, b) Validation of SQS overexpression by western blotting and densitometry. (c) Cell viability after celastrol treatment. (d-f) Intracellular iron, MDA, and GSH levels. Data represent mean $\pm$ SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. DMSO; & $P < 0.05$, && $P < 0.01$, &&& $P < 0.001$ vs. respective celastrol-treated group.

3.4. Clinical Transcriptomic Context from MMRF-COMPASS

To place the *in vitro* findings in a clinical context, we analyzed the MMRF-COMPASS cohort ($n = 764$ primary bone marrow $CD138^+$ plasma cell samples). FDFT1 expression did not differ significantly across ISS stages (Kruskal-Wallis $P = 0.068$) and was not associated with overall survival in univariate (HR = 1.064, 95% CI: 0.908–1.247, $P = 0.441$) or multivariate Cox regression adjusted for age, sex, and ISS stage (HR = 1.059, $P = 0.482$). These findings indicate that FDFT1 is not an independent prognostic biomarker in MM, consistent with its role as a metabolic enzyme supporting ferroptosis defense rather than a driver of disease progression.

Pearson correlation analysis revealed significant associations between FDFT1 and ferroptosis core genes after BH correction (Table 1; Figure 4a). Key findings include a positive correlation with FSP1/AIFM2 ($r = 0.135$, FDR = 0.001), a negative correlation with IREB2/IRP2 ($r = -0.240$, FDR < 0.001), the iron regulatory protein that promotes iron uptake and ferroptosis sensitivity, and the strongest positive correlation with GSR (glutathione reductase; $r = 0.425$, FDR < 0.001), linking MVA pathway activity to glutathione homeostasis. This pattern — positive with FSP1 and GSR, negative with IREB2 — is consistent with a model in which elevated SQS expression supports a ferroptosis-resistant cellular state.

GSVA/ssGSEA identified significant pathway activity differences between FDFT1-high and FDFT1-low tumors (Table 1; Figure 4b). Ferroptosis defense was elevated in FDFT1-high tumors ($P_{\text{adj}} = 1.37 \times 10^{-6}$), as was the ferroptosis core gene set ($P_{\text{adj}} = 1.7 \times 10^{-4}$), while the ferroptosis induction pathway was not significantly different ($P_{\text{adj}} = 0.364$). GSEA confirmed ferroptosis defense enrichment (NES = 2.13, $P_{\text{adj}} = 2.68 \times 10^{-3}$) and identified oxidative stress response (NES = 2.53), mTORC1 signaling (NES = 2.88), and sterol biosynthesis (NES = 3.03) as top enriched pathways in FDFT1-high tumors (Figure 4c, d). The convergence of GSVA and GSEA — both showing ferroptosis defense but not induction elevated in FDFT1-high tumors — provides cohort-level support for the hypothesis that SQS supports ferroptosis defense in MM.

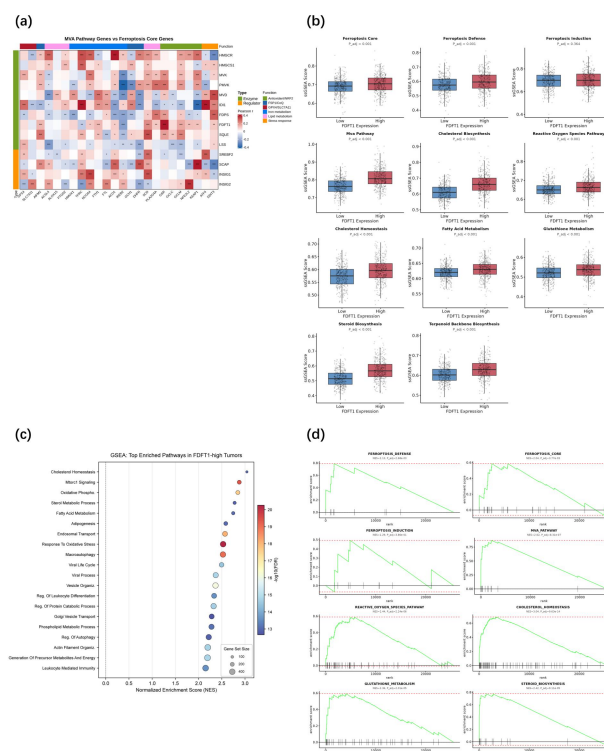


Figure 4. Clinical transcriptomic analysis of FDFT1 in MMRF-COMPASS ($n = 764$). (a) Correlation heatmap: MVA pathway genes vs. ferroptosis core genes. (b) GSVA pathway comparison: FDFT1-high vs. FDFT1-low (selected pathways). (c) GSEA enrichment dotplot for top enriched pathways. (d) GSEA enrichment plot for the ferroptosis defense gene set.

Table 1. FDFT1-ferroptosis gene correlations and pathway activity in MMRF-COMMPASS (n = 764)

Gene / Pathway	r or NES	P value	FDR (BH)
Correlation (Pearson r)			
GSR (glutathione reductase)	+0.425	6.83×10^{-35}	< 0.001
FSP1/AIFM2	+0.135	1.93×10^{-4}	0.001
IREB2 (IRP2)	-0.240	1.93×10^{-11}	< 0.001
GPX4	+0.060	0.096	0.145 (NS)
SLC7A11	-0.051	0.161	0.228 (NS)
GSVA pathway (P _{adj})			
Ferroptosis defense	-	-	1.37×10^{-6}
Ferroptosis core	-	-	1.7×10^{-4}
Ferroptosis induction	-	-	0.364 (NS)
GSEA (NES)			
Ferroptosis defense	2.13	-	2.68×10^{-3}
Oxidative stress response	2.53	-	5.47×10^{-21}
mTORC1 signaling	2.88	-	3.72×10^{-20}
Sterol biosynthesis	3.03	-	3.94×10^{-13}

NS, not significant. Full correlation matrix and GSVA results are provided in Supplementary Materials.

4. Discussion

In this study, we demonstrate that celastrol exerts anti-MM efficacy by inducing a regulated cell death cascade with a robust ferroptotic component, and that this process is associated with suppression of the MVA-SQS lipid defense axis. Through a combination of phenotypic characterization, pharmacological rescue, genetic overexpression, and clinical transcriptomic analysis, we identify SQS as a functional modulator of celastrol responsiveness in MM.

Ferroptosis is characterized by catastrophic lipid ROS accumulation resulting from failure of cellular antioxidant defense systems, primarily governed by the System Xc-GSH-GPX4 axis and the parallel FSP1-CoQ10 system [7-12]. Our results show that celastrol exposure in MM cells leads to iron overload, GSH depletion, MDA accumulation, and ROS elevation, all of which are attenuated by the ferroptosis inhibitors Fer-1 and DFOM. Importantly, SQS protein levels were downregulated upon celastrol exposure, and this downregulation was partially reversed by ferroptosis inhibitors. These observations place SQS within the ferroptotic cascade, although whether its suppression represents an upstream trigger or a secondary consequence of oxidative injury warrants further investigation.

The functional relevance of SQS downregulation is supported by genetic rescue experiments. Enforced SQS overexpression in RPMI-8226 cells was sufficient to partially attenuate celastrol-induced cytotoxicity, reduce intracellular iron and MDA accumulation, and preserve GSH levels. SQS catalyzes the first committed step in sterol biosynthesis within

the MVA pathway [21,22]. MVA-derived metabolites, including CoQ10 and squalene, have been shown to reinforce ferroptosis defense [23-25]. It is therefore plausible that SQS suppression by celastrol compromises the cellular pool of these protective intermediates, thereby lowering the threshold for lipid peroxidation. The FSP1-CoQ10 axis [11,12] and DHODH-mediated mitochondrial defense [13] represent parallel ferroptosis-suppressing systems that may also be affected when MVA pathway flux is disrupted. Interestingly, the relationship between MVA pathway perturbation and ferroptosis appears context-dependent: squalene accumulation protects certain solid tumor cells and lymphomas from oxidative cell death [23], whereas in our hematological model, SQS downregulation is associated with enhanced ferroptotic sensitivity.

The downregulation of SREBP2 observed in our western blot data is notable, as SREBP2 is the master transcriptional regulator of MVA pathway genes including FDFT1. Celastrol has previously been reported to modulate SREBP signaling in other cancer contexts [27,28]. It is therefore possible that celastrol suppresses SQS expression at least in part through SREBP2 downregulation, rather than through direct physical interaction with the SQS protein. This transcriptional mechanism would be consistent with the observed parallel downregulation of GPX4, SLC7A11, and FSP1, which are known targets of SREBP2 and related lipid metabolic regulatory networks. We acknowledge that the present study does not establish a direct physical interaction between celastrol and SQS, and the causal chain linking celastrol, SREBP2, SQS, and ferroptosis requires further mechanistic dissection.

The clinical transcriptomic analysis of the MMRF-COMMPASS cohort (n = 764) provides translational context. FDFT1 expression was not independently associated with overall survival, indicating that SQS is not a prognostic biomarker but rather a functional metabolic target. This is not surprising, as many validated drug targets are not themselves prognostic markers; their therapeutic relevance lies in druggability and functional contribution to resistance phenotypes. Critically, FDFT1-high tumors showed significantly elevated ferroptosis defense pathway activity but not ferroptosis induction pathway activity, providing cohort-level evidence that SQS expression is associated with a ferroptosis-resistant state in MM. The positive correlation between FDFT1 and FSP1 (r = 0.135) and GSR (r = 0.425), together with the negative correlation with IREB2 (r = -0.240), connect MVA pathway activity to both the glutathione-

dependent and glutathione-independent ferroptosis defense arms, as well as to iron regulatory signaling.

Several limitations should be acknowledged. First, the present study is restricted to in vitro models; rigorous in vivo validation using human MM xenograft or immunocompetent models will be essential to establish translational relevance. Second, loss-of-function experiments (SQS knockdown or knockout) and pharmacological controls with known SQS inhibitors such as zaragozic acid A or lapaquistat would further strengthen the causal evidence. Third, the downstream metabolic consequences of SQS suppression—particularly regarding carbon flux redistribution within the MVA pathway and direct measurement of CoQ10, squalene, and cholesterol depletion—remain to be elucidated using stable-isotope-resolved metabolomics. Fourth, the mechanism by which celastrol downregulates SQS and SREBP2, whether through direct transcriptional modulation, post-translational effects, or upstream signaling interference, requires further investigation. Fifth, while the MMRF-COMMPASS analysis provides correlative clinical support, prospective validation in independent cohorts is needed.

From a translational perspective, our findings position the MVA-SQS axis as a candidate metabolic vulnerability in MM. Given that statins are clinically used, and SQS inhibitors represent a promising repurposing strategy [41,42], repurposing these agents or developing SQS-selective inhibitors in combination with established anti-MM therapies represents a rational future direction. The observation that celastrol, a natural product with known anti-inflammatory and anti-tumor activities [27-30], can modulate this axis further supports the exploration of MVA-targeting strategies for MM treatment.

5. Conclusions

Our study demonstrates that celastrol induces ferroptosis-associated cell death in multiple myeloma cells, and that this process is functionally associated with suppression of the MVA-SQS lipid defense axis. Pharmacological rescue with ferroptosis inhibitors, genetic overexpression of SQS, and clinical transcriptomic evidence from the MMRF-COMMPASS cohort collectively support SQS as a ferroptosis defense node whose modulation influences celastrol sensitivity in MM. These findings provide a preclinical rationale for further investigation of SQS-targeted strategies to overcome drug resistance in MM.

Acknowledgments: Not applicable.

Ethics approval and consent to participate: Not applicable.

Clinical trial number: Not applicable.

Availability of data and materials: The MMRF-COMMPASS dataset used in this study is publicly available via the GDC Data Portal (<https://portal.gdc.cancer.gov/>). All other data supporting the results of this study are included within the article and its supplementary materials.

Competing interests: The authors declare no conflicts of interest.

Funding: This research was funded by the Shanghai Pudong New Area Science, Technology and Economy Commission, grant number PKJ2024-Y68.

Authors' contributions: Methodology, validation, investigation, and writing—original draft preparation, H.Y.; conceptualization, supervision, funding acquisition, writing—review and editing, B.P. All authors have read and agreed to the published version of the manuscript.

References

- [1] Malard, F.; Neri, P.; Bahlis, N. J.; Terpos, E.; Moukalled, N.; Hungria, V. T. M.; Manier, S.; Mohty, M. Multiple myeloma. *Nat Rev Dis Primers* **2024**, *10*, 45. DOI: 10.1038/s41572-024-00529-7.
- [2] Scalzulli, E.; Grammatico, S.; Vozella, F.; Petrucci, M. T. Proteasome inhibitors for the treatment of multiple myeloma. *Expert Opin Pharmacother* **2018**, *19*, 375-386. DOI: 10.1080/14656566.2018.1441287.
- [3] Garfall, A. L. New Biological Therapies for Multiple Myeloma. *Annu Rev Med* **2024**, *75*, 13-29. DOI: 10.1146/annurev-med-050522-033815.
- [4] Gertz, M. A. Is There Still a Role for Stem Cell Transplantation in Multiple Myeloma?. *Hematol Oncol Clin North Am* **2024**, *38*, 407-420. DOI: 10.1016/j.hoc.2023.12.005.
- [5] Forster, S.; Radpour, R.; Ochsenbein, A. F. Molecular and immunological mechanisms of clonal evolution in multiple myeloma. *Front Immunol* **2023**, *14*, 1243997. DOI: 10.3389/fimmu.2023.1243997.
- [6] Neri, P.; Leblay, N.; Lee, H.; Gulla, A.; Bahlis, N. J.; Anderson, K. C. Just scratching the surface: novel treatment approaches for multiple myeloma targeting cell membrane proteins. *Nat Rev Clin Oncol* **2024**, *21*, 590-609. DOI: 10.1038/s41571-024-00913-y.
- [7] Dixon, S. J.; Olzmann, J. A. The cell biology of ferroptosis. *Nat Rev Mol Cell Biol* **2024**, *25*, 424-442. DOI: 10.1038/s41580-024-00703-5.

- [8] Stockwell, B. R.; Friedmann Angeli, J. P.; Bayir, H.; Bush, A. I.; Conrad, M.; Dixon, S. J.; Fulda, S.; Gascón, S.; Hatzios, S. K.; Kagan, V. E.; Noel, K.; Jiang, X.; Linkermann, A.; Murphy, M. E.; Overholtzer, M.; Oyagi, A.; Pagnussat, G. C.; Park, J.; Ran, Q.; Rosenfeld, C. S.; Salnikow, K.; Tang, D.; Torti, F. M.; Torti, S. V.; Toyokuni, S.; Woerpel, K.; Zhang, D. D. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell* **2017**, *171*, 273-285. DOI: 10.1016/j.cell.2017.09.021.
- [9] Lei, G.; Zhuang, L.; Gan, B. Targeting ferroptosis as a vulnerability in cancer. *Nat Rev Cancer* **2022**, *22*, 381-396. DOI: 10.1038/s41568-022-00459-0.
- [10] Zheng, J.; Conrad, M. Ferroptosis: when metabolism meets cell death. *Physiol Rev* **2025**, *105*, 651-706. DOI: 10.1152/physrev.00031.2024.
- [11] Bersuker, K.; Hendricks, J. M.; Li, Z.; Magtanong, L.; Ford, B.; Tang, P. H.; Roberts, M. A.; Tong, B.; Maimone, T. J.; Zoncu, R.; Bassik, M. C.; Nomura, D. K.; Dixon, S. J.; Olzmann, J. A. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* **2019**, *575*, 688-692. DOI: 10.1038/s41586-019-1705-2.
- [12] Doll, S.; Freitas, F. P.; Shah, R.; Aldrovandi, M.; da Silva, M. C.; Ingold, I.; Goya Grocin, A.; Xavier da Silva, T. N.; Panzilius, E.; Scheel, C. H.; Mourão, A.; Buday, K.; Sato, M.; Wanninger, J.; Vignane, T.; Mohana, V.; Rehberg, M.; Flatley, A.; Schepers, A.; Kurz, A.; White, D.; Sauer, M.; Sattler, M.; Tate, E. W.; Schmitz, W.; Schulze, A.; O'Donnell, V.; Proneth, B.; Popowicz, G. M.; Pratt, D. A.; Angeli, J. P. F.; Conrad, M. FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* **2019**, *575*, 693-698. DOI: 10.1038/s41586-019-1707-0.
- [13] Mao, C.; Liu, X.; Zhang, Y.; Lei, G.; Yan, Y.; Lee, H.; Koppula, P.; Wu, S.; Zhuang, L.; Fang, B.; Poyurovsky, M. V.; Olszewski, K.; Gan, B. DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature* **2021**, *593*, 586-590. DOI: 10.1038/s41586-021-03539-7.
- [14] Campanella, A.; Santambrogio, P.; Fontana, F.; Frenquelli, M.; Cenci, S.; Marcatti, M.; Sitia, R.; Tonon, G.; Camaschella, C. Iron increases the susceptibility of multiple myeloma cells to bortezomib. *Haematologica* **2013**, *98*, 971-979. DOI: 10.3324/haematol.2012.074872.
- [15] Camiolo, G.; Barbato, A.; Giallongo, C.; Vicario, N.; Romano, A.; Parrinello, N. L.; Parenti, R.; Sandoval, J. C.; García-Moreno, D.; Lazzarino, G.; Avola, R.; Palumbo, G. A.; Mulero, V.; Li Volti, G.; Tibullo, D.; Di Raimondo, F. Iron regulates myeloma cell/macrophage interaction and drives resistance to bortezomib. *Redox Biol* **2020**, *36*, 101611. DOI: 10.1016/j.redox.2020.101611.
- [16] Xian, M.; Wang, Q.; Xiao, L.; Zhong, L.; Xiong, W.; Ye, L.; Su, P.; Zhang, C.; Li, Y.; Orłowski, R. Z.; Zhan, F.; Ganguly, S.; Zu, Y.; Qian, J.; Yi, Q. Leukocyte immunoglobulin-like receptor B1 (LILRB1) protects human multiple myeloma cells from ferroptosis by maintaining cholesterol homeostasis. *Nat Commun* **2024**, *15*, 5767. DOI: 10.1038/s41467-024-50073-x.
- [17] Zhang, J.; Liu, Y.; Zuo, L.; Fan, F.; Yan, H.; Zhao, F.; Li, J.; Ma, C.; Li, Q.; Xu, A.; Xu, J.; Zhang, B.; Hu, Y.; Sun, C. Class II ferroptosis inducers are a novel therapeutic approach for t(4;14)-positive multiple myeloma. *Blood Adv* **2024**, *8*, 5022-5038. DOI: 10.1182/bloodadvances.2023010335.
- [18] Jiang, H.; Wang, L.; Zhang, Q.; Wang, S.; Jia, L.; Cheng, H.; Wang, J.; Li, X.; Xie, Y.; Wang, Y.; Hu, M.; Guo, J.; Li, Q.; Peng, Z.; Wang, M.; Xie, Y.; Li, T.; Wang, Y.; Geng, B. D.; Swaminathan, S.; Bergsagel, P. L.; Liu, Z. Bone marrow stromal cells dictate lanosterol biosynthesis and ferroptosis of multiple myeloma. *Oncogene* **2024**, *43*, 1644-1653. DOI: 10.1038/s41388-024-03020-5.
- [19] Guerra, B.; Recio, C.; Aranda-Tavío, H.; Guerra-Rodríguez, M.; García-Castellano, J. M.; Fernández-Pérez, L. The Mevalonate Pathway, a Metabolic Target in Cancer Therapy. *Front Oncol* **2021**, *11*, 626971. DOI: 10.3389/fonc.2021.626971.
- [20] Xue, L.; Qi, H.; Zhang, H.; Ding, L.; Huang, Q.; Zhao, D.; Wu, B. J.; Li, X. Targeting SREBP-2-Regulated Mevalonate Metabolism for Cancer Therapy. *Front Oncol* **2020**, *10*, 1510. DOI: 10.3389/fonc.2020.01510.
- [21] Chang, H.; Cheng, T.; Wang, A. H. Structure, catalysis, and inhibition mechanism of prenyltransferase. *IUBMB Life* **2021**, *73*, 40-63. DOI: 10.1002/iub.2418.
- [22] Mavridi, D.; Katavati, T.; Kourounakis, A. P. The Contribution of Cholesterol and Squalene Synthase in Cancer: Molecular Mechanisms, Lipid Rafts and Therapeutic Approaches. *Med Res Rev* **2025**, *46*, 352-381. DOI: 10.1002/med.70012.
- [23] Garcia-Bermudez, J.; Baudrier, L.; Bayraktar, E. C.; Shen, Y.; La, K.; Guarecuco, R.; Yucel, B.; Fiore, D.; Tavora, B.; Freinkman, E.; Chan, S. H.; Lewis, C.; Min, W.; Inghirami, G.; Sabatini, D. M.; Birsoy, K. Squalene accumulation in cholesterol auxotrophic lymphomas prevents oxidative cell death. *Nature* **2019**, *567*, 118-122. DOI: 10.1038/s41586-019-0945-5.

- [24] Sun, Q.; Liu, D.; Cui, W.; Cheng, H.; Huang, L.; Zhang, R.; Gu, J.; Liu, S.; Zhuang, X.; Lu, Y.; Chu, B.; Li, J. Cholesterol mediated ferroptosis suppression reveals essential roles of Coenzyme Q and squalene. *Commun Biol* **2023**, *6*, 1108. DOI: 10.1038/s42003-023-05477-8.
- [25] Tosi, G.; Paoli, A.; Zuccolotto, G.; Turco, E.; Simonato, M.; Tosoni, D.; Tucci, F.; Lugato, P.; Giomo, M.; Elvassore, N.; Rosato, A.; Cogo, P.; Pece, S.; Santoro, M. M. Cancer cell stiffening via CoQ10 and UBIAD1 regulates ECM signaling and ferroptosis in breast cancer. *Nat Commun* **2024**, *15*, 8214. DOI: 10.1038/s41467-024-52523-y.
- [26] Yang, H.; Wang, J.; Xie, J.; Yang, H.; Wu, X. Molecular mechanisms and delivery strategies of celastrol targeting inflammation-associated autoimmune diseases. *J Ethnopharmacol* **2026**, *355*, 120719. DOI: 10.1016/j.jep.2025.120719.
- [27] Jiang, Q.; Liu, Z.; Cheng, J.; Geng, Z.; Gu, Y.; Wei, Y.; Yu, M.; Bi, Y. The Antitumor Potential of Celastrol: Research Progress on Antitumor Mechanisms and Strategies for Toxicity Reduction with Efficacy Enhancement. *Biomolecules* **2026**, *16*, 620. DOI: 10.3390/biom16050620.
- [28] Lu, Y.; Liu, Y.; Zhou, J.; Li, D.; Gao, W. Biosynthesis, total synthesis, structural modifications, bioactivity, and mechanism of action of the quinone-methide triterpenoid celastrol. *Med Res Rev* **2021**, *41*, 1022-1060. DOI: 10.1002/med.21751.
- [29] Shanmugam, M. K.; Ahn, K. S.; Lee, J. H.; Kannaiyan, R.; Mustafa, N.; Manu, K. A.; Siveen, K. S.; Sethi, G.; Chng, W. J.; Kumar, A. P. Celastrol Attenuates the Invasion and Migration and Augments the Anticancer Effects of Bortezomib in a Xenograft Mouse Model of Multiple Myeloma. *Front Pharmacol* **2018**, *9*, 365. DOI: 10.3389/fphar.2018.00365.
- [30] Zhong, Y.; Xu, G.; Huang, S.; Zhao, L.; Zeng, Y.; Xiao, X.; An, J.; Liu, J.; Yang, T. Celastrol induce apoptosis of human multiple myeloma cells involving inhibition of proteasome activity. *Eur J Pharmacol* **2019**, *853*, 184-192. DOI: 10.1016/j.ejphar.2019.03.036.
- [31] Ding, Y.; Bai, Y.; Chen, T.; Chen, S.; Feng, W.; Ma, S.; Zhang, Q. Disruption of the sorcin-PAX5 protein-protein interaction induces ferroptosis by promoting the FBXL12-mediated ubiquitination of ALDH1A1 in pancreatic cancer. *J Hematol Oncol* **2025**, *18*, 27. DOI: 10.1186/s13045-025-01680-8.
- [32] Liu, M.; Fan, Y.; Li, D.; Han, B.; Meng, Y.; Chen, F.; Liu, T.; Song, Z.; Han, Y.; Huang, L.; Chang, Y.; Cao, P.; Nakai, A.; Tan, K. Ferroptosis inducer erastin sensitizes NSCLC cells to celastrol through activation of the ROS-mitochondrial fission-mitophagy axis. *Mol Oncol* **2021**, *15*, 2084-2105. DOI: 10.1002/1878-0261.12936.
- [33] Luo, P.; Liu, D.; Zhang, Q.; Yang, F.; Wong, Y.-K.; Xia, F.; Zhang, J.; Chen, J.; Tian, Y.; Yang, C.; Dai, L.; Shen, H.-M.; Wang, J. Celastrol induces ferroptosis in activated HSCs to ameliorate hepatic fibrosis via targeting peroxiredoxins and HO-1. *Acta Pharm Sin B* **2022**, *12*, 2300-2314. DOI: 10.1016/j.apsb.2021.12.007.
- [34] Colaprico, A.; Silva, T. C.; Olsen, C.; Garofano, L.; Cava, C.; Garolini, D.; Sabedot, T. S.; Malta, T. M.; Pagnotta, S. M.; Castiglioni, I.; Ceccarelli, M.; Bontempi, G.; Noushmehr, H. TCGAAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res* **2016**, *44*, e71. DOI: 10.1093/nar/gkv1507.
- [35] Love, M. I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **2014**, *15*, 550. DOI: 10.1186/s13059-014-0550-8.
- [36] Therneau, T. M. survival: A Package for Survival Analysis in R. *R package version 3.5-5*; 2023. Available online: <https://CRAN.R-project.org/package=survival>.
- [37] Hänzelmann, S.; Castelo, R.; Guinney, J. GSVA: gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics* **2013**, *14*, 7. DOI: 10.1186/1471-2105-14-7.
- [38] Dolgalev, I. msigdb: MSigDB Gene Sets for Multiple Organisms in a Tidy Data Format. *R package version 7.5.1*; 2022. Available online: <https://CRAN.R-project.org/package=msigdb>.
- [39] Ritchie, M. E.; Phipson, B.; Wu, D.; Hu, Y.; Law, C. W.; Shi, W.; Smyth, G. K. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* **2015**, *43*, e47. DOI: 10.1093/nar/gkv007.
- [40] Korotkevich, G.; Sukhov, V.; Budin, N.; Shpak, B.; Artyomov, M. N.; Sergushichev, A. Fast gene set enrichment analysis. *bioRxiv* **2016**, DOI: 10.1101/060012.
- [41] Longo, J.; van Leeuwen, J. E.; Elbaz, M.; Branchard, E.; Penn, L. Z. Statins as Anticancer Agents in the Era of Precision Medicine. *Clin Cancer Res* **2020**, *26*, 5791-5800. DOI: 10.1158/1078-0432.CCR-20-1967.
- [42] Kourounakis, A. P.; Bavavea, E. New applications of squalene synthase inhibitors: Membrane cholesterol as a

therapeutic target. *Arch Pharm (Weinheim)* **2020**, 353, 2000085. DOI: 10.1002/ardp.202000085.