Stearoyl CoA Desaturase is Essential for Regulation of Endoplasmic Reticulum Homeostasis and Tumor Growth in Glioblastoma Cancer Stem Cells

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Highlights

• SCD1 is essential for GSC maintenance and confers tumor growth advantage in vivo

• Therapeutic targeting of SCD1 is highly effective in GBM mouse models

• SCD1 exerts a cytoprotective function in GSCs by regulating ER homeostasis

• ER stress promotes lipogenesis through the activation of IRE1-SREBP1-SCD1 axis
Effects of SCD1-mediated desaturation of FAs

- Cancer cells often require de novo fatty acid synthesis

- **Stearoyl-CoA desaturase-1 (SCD1/SCD)** is important for lipid homeostasis and biosynthesis of monounsaturated fatty acids
SCD1 Is Essential for GSC Maintenance and Tumor Initiation

(A) Mice bearing 83-Fluc GSC tumors received daily injections over 7 days of BSA or PA (3 mg/kg of body weight). Representative brain sections were stained with BODIPY (red) and DAPI (blue). Asterisks depict the tumor injection site.

(B) Immunostaining for NESTIN, SOX2, and nuclei (DAPI) in 19-GSCs expressing shSCR or shSCD1 at day 7 after shRNA transduction. Scale bar, 100 mm.

(C) Immunoblot analysis of SCD1, SOX2, and NESTIN in GSCs expressing shSCR or shSCD1.

(D) Relative expression of stem cell markers determined by qPCR in GSCs transduced with shSCR or shSCD1 for 7 days.

(E) Stem cell frequency in 157 GSCs expressing shSCR or shSCD1 determined using the limited dilution analysis algorithm.

(F) Cell viability in three GSCs, NHA, and NSC expressing shSCR or shSCD1, at day 7 after transduction. Data is expressed as percentage of shSCR.

(G) GSCs (326 and 1123) expressing shSCR or shSCD1 were cultured in the presence of BSA or OA (50 mM). Cell viability was determined 7 days post transduction.

(H) 83-Fluc GSCs transduced with shCtrl or shSCD1 and intracranially implanted in mice (shCtrl, n = 4; shSCD1, n = 6) after 24 h. Survival analysis is shown using Kaplan-Meier curves. p = 0.0025 (two-sided log-rank test). Representative Fluc imaging of brain tumors at day 10 post implantation is also shown.

(I) High-passage 157-Fluc GSCs (5 3 104) expressing control (Ctrl) or SCD1 (SCD1-OE) were implanted in the brain of nude mice (n= 5/group). Longitudinal Fluc imaging shown for individual mice in each group.

(J) H&E staining of brain sections of representative mice from both groups at day 135 post implantation.
Pharmacological Targeting of SCD1 Depletes GSCs

(A) Fold change in caspase-3/7 activation in 326 GSCs treated with CAY (200 nM).

(B) Immunostaining for γ-H2AX in CAY-treated GSCs. Scale bar, 200 mm. Representative images of single nuclei depicting γ-H2AX foci are also shown (inset).

(C) Heatmap representing the quantitative ratio of SFAs and UFAs in 83 CAY-treated GSCs relative to the untreated control. Values below or above 1 are indicative of decreased or increased fatty acids ratios, respectively.

(D) Cell viability in 83-GSCs treated with CAY (100 nM) in the presence of the indicated fatty acids.

(E) Cell viability at day 4 in ten GSC specimens treated with CAY at the indicated doses.

(F) Cell viability and representative bright-field micrographs of secondary spheres at day 9 in 157-GSCs treated with CAY (100 nM).

*p < 0.05, **p < 0.001, Student’s t test.
Therapeutic Targeting of SCD1 in Preclinical GBM Mouse Models

(A) Overview of experimental setup.

(B–D) Mice implanted with 83-Fluc GSCs (2×10⁴; n=8/group) received a daily intranasal dose of DMSO (Control) or CAY (5 mg/kg) for 10 days.

(B) Overtime monitoring of tumor growth with Flucimaging in individual mice from Ctrl and CAY-treated groups. Hash depicts the time of death due to tumor burden.

(C) Ki67 immunostaining in one Ctrl and one CAY-treated mouse. Scale bar, 100 mm.

(D) Kaplan-Meier curves showing median survival in both groups (*p = 0.008, two-sided log-rank test).

(E–G) Mice implanted with 157-Fluc GSCs (1×10⁵; n=8/group) were treated with vehicle or CAY (5 mg/kg).

(E) Overtime Fluc imaging demonstrates the absence of tumor growth in all eight CAY-treated mice.

(F) Survival curves in both groups (p = 0.0002; two-sided log-rank test).

(G) The ratio of liver weight to the body weight in both experimental groups is shown. ns, non-significant by Student’s t test.
SCD1 Inhibition Promotes ER Stress and Triggers UPR-Mediated Apoptotic Signaling

(A) Relative mRNA expression of ER stress markers in GSCs treated with CAY (200 nM) for 24 and 48 h.

(B) Immunoblot analysis of four GSCs treated with CAY for 48 h.

(C) Fold change in caspase-3/7 activation after treatment with CAY (50 nM) in the presence or absence of PA (50 mM) or OA (50 mM).

(D) Cell viability in five GSC specimens and NHA, treated with CAY (50 nM) or the combination of CAY and PA (50 mM) for 4 days.

(E) Fold change in caspase-3/7 activation after treatment with CAY (50 nM) in the presence or absence of PBA (5 mM).

(F) Cell viability in GSCs pretreated with CAY for 24 h followed by PBA treatment for 4 days.

(G) Relative mRNA expression of sXBP1 and CHOP in GSCs treated with CAY and/or OA (50 mM).

(H) Immunoblot analysis of phosphorylated c-Jun in GSCs treated with CAY.

(I) Cell viability of GSCs treated with CAY (50 nM) or the combination of CAY with: JNK inhibitor (SP 600125: 20 mM), IRE1 inhibitors (4m8C:25 mM and KIRA6: 5 mM), PERK inhibitor (GSK2656157: 10 mM), and ATF6 inhibitor (Ceapin A7: 20 mM).
ER Stress Promotes a Lipogenic Signature through SREBP1 and IRE1 Signaling

(A) De novo lipogenesis pathway.
(B) Relative mRNA expression of SREBP1 target genes in GSCs treated with Tg (200 nM).
(C–E) Protein expression of SCD1 in GSCs treated with PA and SA (C) or the ER stress inhibitors PBA (2.5, 5, and 10 mM), TUCDA (0.5 mM), and azoramide (50 mM) (D and E).
(F) Immunoblot analysis showing an increased SCD1 expression in four GSCs treated with CAY (200 nM).
(G) Cell viability in GSCs treated with the indicated doses of CAY in combination with T0901317 (25 mM) or SR9243 (10 mM) for 3 days.
(H) Immunoblot analysis of SCD1 expression following IRE1 knockdown.
(I) Relative mRNA expression of SCD1 target genes in GSCs treated with Tg (300 nM) in the presence or absence of IRE1 inhibitor 4m8C.

(A) Citrate → Acetate → Acetyl-CoA → ACCS2 → ACC1 → FASN → ELOVL6 → SCD1
(B) Relative expression of SREBP1 target genes in GSCs treated with Tg (200 nM).
(C) Protein expression of SCD1 in GSCs treated with PA and SA (C) or the ER stress inhibitors PBA (2.5, 5, and 10 mM), TUCDA (0.5 mM), and azoramide (50 mM) (D and E).
(F) Immunoblot analysis showing an increased SCD1 expression in four GSCs treated with CAY (200 nM).
(G) Cell viability in GSCs treated with the indicated doses of CAY in combination with T0901317 (25 mM) or SR9243 (10 mM) for 3 days.
(H) Immunoblot analysis of SCD1 expression following IRE1 knockdown.
(I) Relative mRNA expression of SCD1 target genes in GSCs treated with Tg (300 nM) in the presence or absence of IRE1 inhibitor 4m8C.
SCD1 Inhibition Compromises DNA Damage Repair and Increases Temozolomide Cytotoxicity

(A) Relative mRNA expression of ER stress markers in 83-GSCs treated with CAY (200 nM), PA (200 mM), TMZ (100 mM), or their respective combination as indicated.

(B) Immunoblot analysis of Rad51 and g-H2AX in GSCs treated with CAY and TMZ.

(C) Immunostaining for g-H2AX in 83-GSCs treated with CAY (50 nM) and TMZ (100 mM). Scale bar, 200 mm. Representative images of single nuclei depicting g-H2AX foci are also shown (inset).

(D) Fold change in caspase-3/7 activation in GSCs pretreated with CAY (200 nM) followed by TMZ treatment (100 mM).

(E) GSCs were pretreated with CAY for 24 h prior to TMZ treatment. Cell viability was measured after 5 days. MGG8 were treated with CAY (100 nM) and TMZ (0–10 mM). GSCs (83 and L0) were treated with CAY (10 nM) and TMZ (0–100 mM).

(F and G) MGG23 were pretreated with CAY (100 nM) for 24 h followed by TMZ (100 mM) for 7 days. Secondary spheres were counted 15 days after treatment (F). Micrographs of neurospheres are shown in (G). Scale bar, 100 mm.

(H) Cell viability in 326-GSCs after 7 days of treatment with CAY (10 nM), TMZ (50 mM), or their combination in the presence of OA, PBA, azoramide, PERK, or IRE1 inhibitor.
Summary

Inherent plasticity and various survival cues allow glioblastoma stem-like cells (GSCs) to survive and proliferate under intrinsic and extrinsic stress conditions.

Here, we report that GSCs depend on the adaptive activation of ER stress and subsequent activation of lipogenesis and particularly stearoyl CoA desaturase (SCD1), which promotes ER homeostasis, cytoprotection, and tumor initiation.

1) Pharmacological targeting of SCD1 is particularly toxic due to the accumulation of saturated fatty acids, which exacerbates ER stress, triggers apoptosis, impairs RAD51-mediated DNA repair, and achieves a remarkable therapeutic outcome with 25%–100% cure rate in xenograft mouse models.

2) Mechanistically, divergent cell fates under varying levels of ER stress are primarily controlled by the ER sensor IRE1, which either promotes SCD1 transcriptional activation or converts to apoptotic signaling when SCD1 activity is impaired.

Taken together, the dependence of GSCs on fatty acid desaturation presents an exploitable vulnerability to target glioblastoma.