Pyruvate anaplerosis is a mechanism of resistance to pharmacological glutaminase inhibition in Triple-receptor Negative Breast Cancer

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Abstract

Background

Glutamine serves as an important nutrient with many cancer types displaying glutamine dependence. Following cellular uptake glutamine is converted to glutamate in a reaction catalysed by mitochondrial glutaminase. This glutamate has many uses, including acting as an anaplerotic substrate (via alpha-ketoglutarate) to replenish TCA cycle intermediates. CB-839 is a potent, selective, orally bioavailable glutaminase inhibitor that has activity in Triple receptor-Negative Breast Cancer (TNBC) cell lines and evidence of efficacy in advanced TNBC patients.

Methods

A panel of eleven breast cancer cell lines was used to investigate the anti-proliferative effects of the glutaminase inhibitors CB-839 and BPTES in different types of culture medium, with or without additional pyruvate supplementation. The abundance of the TCA cycle intermediate fumarate was quantified as a measure if TCA cycle anaplerosis. Pyruvate secretion by TNBC cultures was then assessed with or without AZD3965, a monocarboxylate transporter 1 (MCT1) inhibitor. Finally, two dimensional (2D) monolayer and three dimensional (3D) spheroid assays were used to compare the effect of microenvironmental growth conditions on CB-839 activity.

Results

The anti-proliferative activity of CB-839 in a panel of breast cancer cell lines was similar to published reports, but with a major caveat; growth inhibition by CB-839 was strongly attenuated in culture medium containing pyruvate. This pyruvate-dependent attenuation was also observed with a related glutaminase inhibitor, BPTES. Studies demonstrated that exogenous pyruvate acted as an anaplerotic substrate preventing the decrease of fumarate in CB-839-treated conditions. Furthermore, endogenously produced pyruvate secreted by TNBC cell lines was able to act in a paracrine manner to significantly decrease the sensitivity of recipient cells to glutaminase inhibition. Suppression of pyruvate secretion using the MCT1 inhibitor AZD3965, antagonised this paracrine effect and increased CB-839 activity. Finally, CB-839 activity was significantly compromised in 3D compared with 2D TNBC culture models, suggesting that 3D microenvironmental features impair glutaminase

inhibitor responsiveness.

Conclusion

This study highlights the potential influence that both circulating and tumour-derived pyruvate can have on glutaminase inhibitor efficacy. Furthermore, it highlights the benefits of 3D spheroid cultures to model the features of the tumour microenvironment and improve the in vitro investigation of cancer metabolism-targeted therapeutics.

Full Text

Supplementary Table Caption

Supplementary Table S1: Comparison of RPMI 1640, αMEM and DMEM culture medium formulation.

Figures
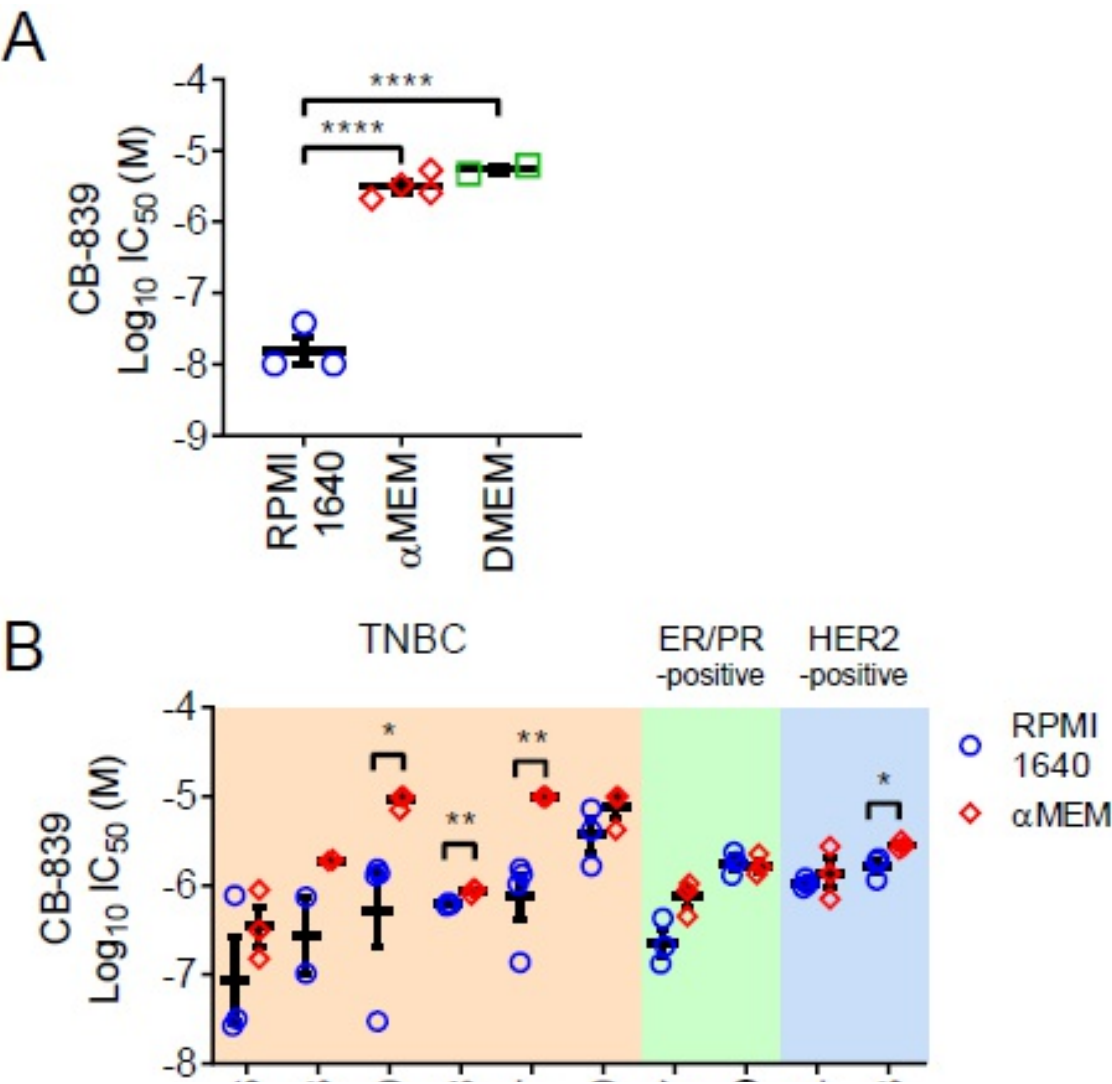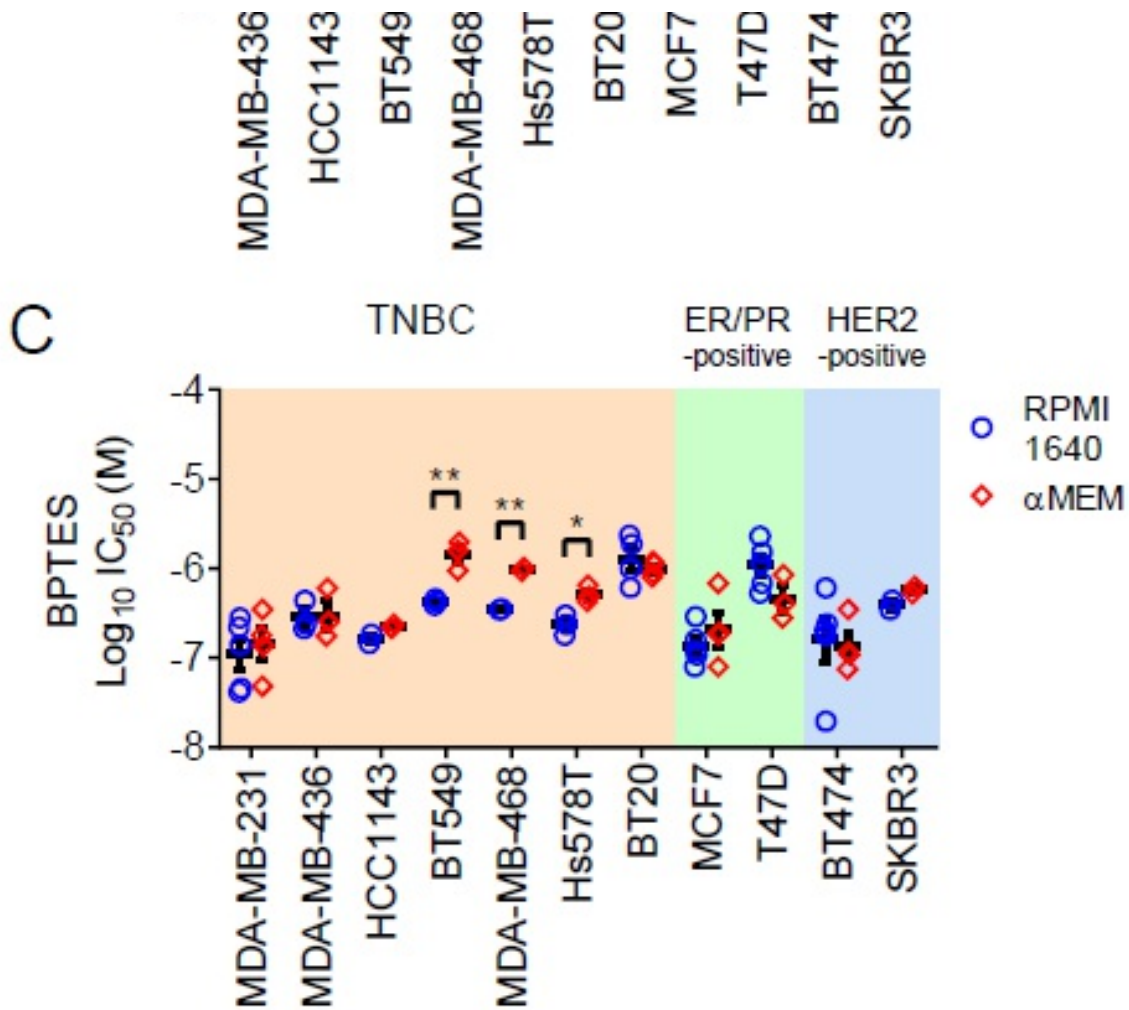
Figure 1

Breast cancer cell lines display differences in sensitivity to pharmacological glutaminase inhibition depending on culture medium composition. A MDA-MB-231 cells were more sensitive to 3 days CB-839 exposure when assayed in RPMI 1640 + 5% FBS (RPMI 1640) compared with αMEM + 5% FBS (αMEM) or DMEM + 5% FBS (DMEM) (mean ± SEM, n = 2-4, ANOVA). CB- 839 (B) or BPTES (C) display more potent IC50 values when assayed in RPMI 1640 + 5% FBS compared with αMEM + 5% FBS in many breast cancer cell lines (mean ± SEM, n = 2-4, unpaired t test).
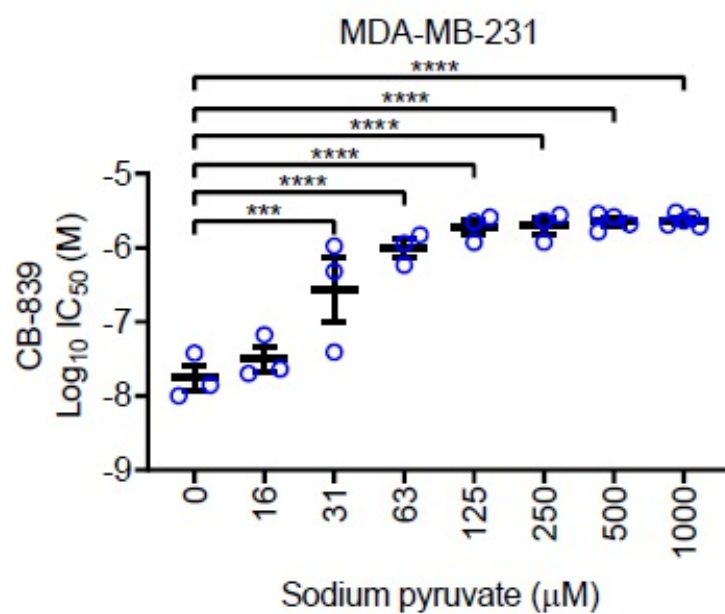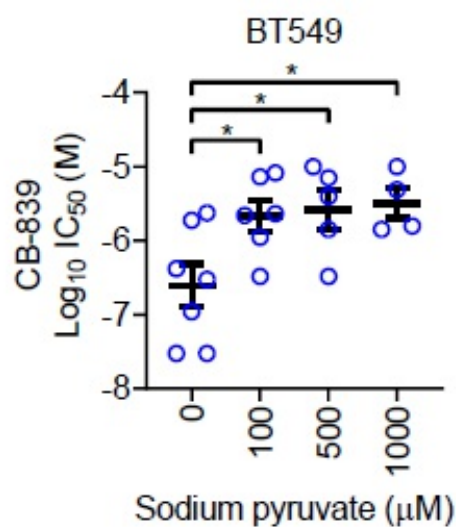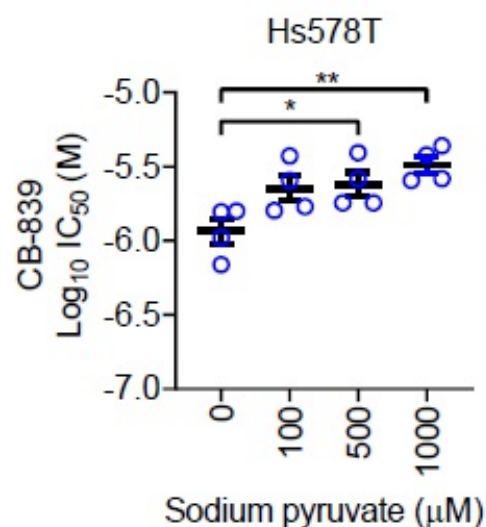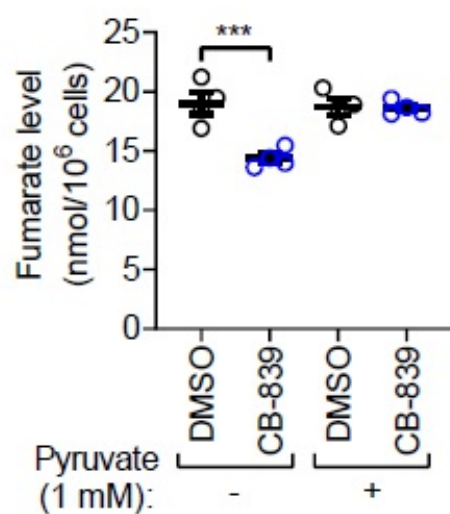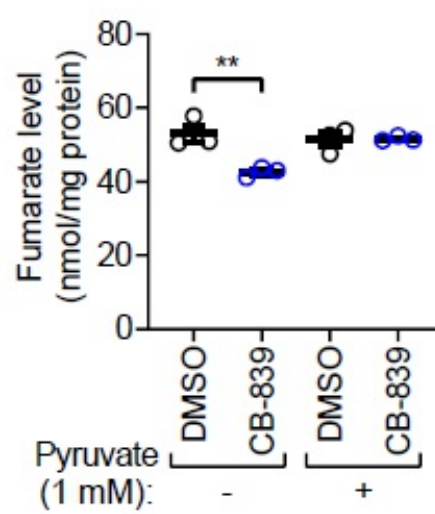
Figure 2

Pyruvate impairs sensitivity to glutaminase inhibition by increasing TCA cycle anaplerosis. Increasing sodium pyruvate concentration in RPMI 1640 + 5% FBS increases CB-839 IC50 (mean ± SEM, n = 3-7, ANOVA) in A MDA-MB-231, B BT549 or C Hs578T cells during a 3 day assay. 24 h treatment with CB-839 decreased fumarate level in MDA-MB-231 cells in RPMI 1640 + 5% FBS but not RPMI 1640 + 5% FBS supplemented with 1 mM sodium pyruvate. Fumarate level was normalised to either initial cell number seeded (D) or protein amount at endpoint (E) (mean ± SEM, n = 3-4, ANOVA).
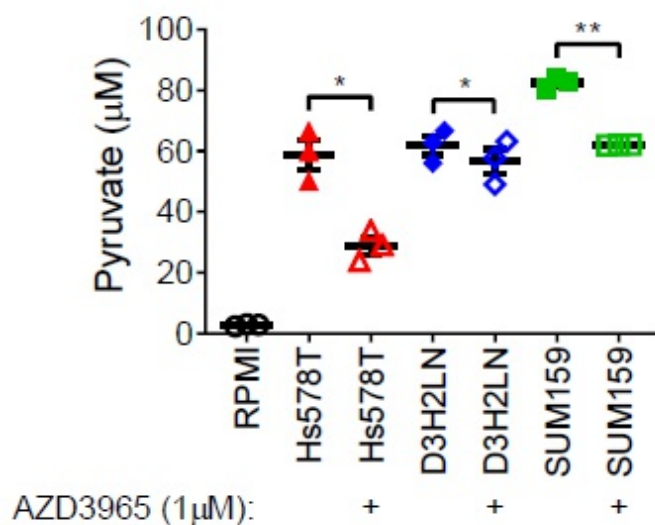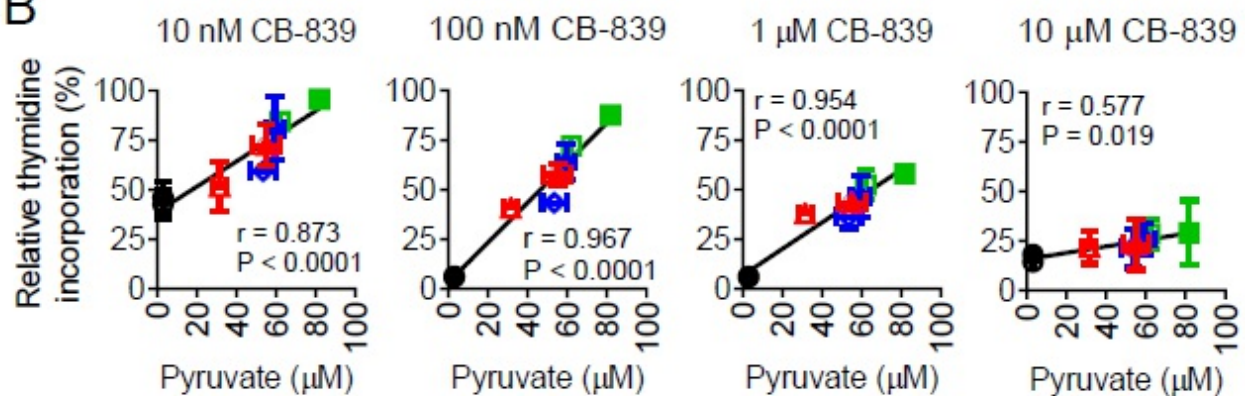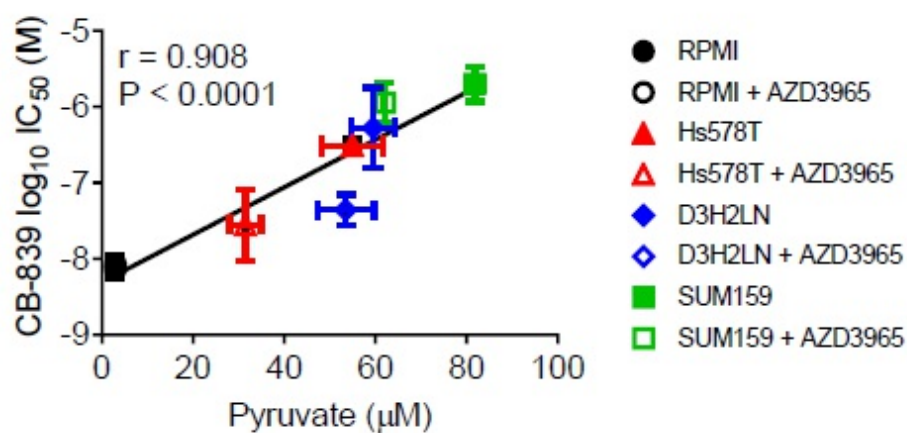
Figure 3

Pyruvate secreted by TNBC cell lines reduces the potency of CB-839. A Pharmacological

MCT1 inhibition using 1 μM AZD3965 reduced the secretion of pyruvate by Hs578T and

SUM159PT (SUM159) but not MDA-MB-231-luc-D3H2LN (D3H2LN) cells. Pyruvate

concentration in the conditioned or unconditioned RPMI 1640 + 5% FBS (RPMI) culture medium was quantified after 48 h incubation (mean ± SEM, n = 3, unpaired test). B The pyruvate concentration in the conditioned culture medium from A correlates with resistance of recipient MDA-MB-231 cells to CB-839-treatment at 10 nM, 100 nM, 1 µM or 10 µM over 3 days exposure. For each of the TNBC cell lines studied the AZD3965-treated samples of conditioned culture medium demonstrated a decrease in relative thymidine incorporation in recipient MDA-MB-231 cells (mean ± SD, n = 2). C IC50 analysis also demonstrates a correlation between CB-839 sensitivity and pyruvate concentration in the conditioned culture medium from A (mean ± SD, n = 2). Correlations were computed by Pearson r correlation coefficient analysis.
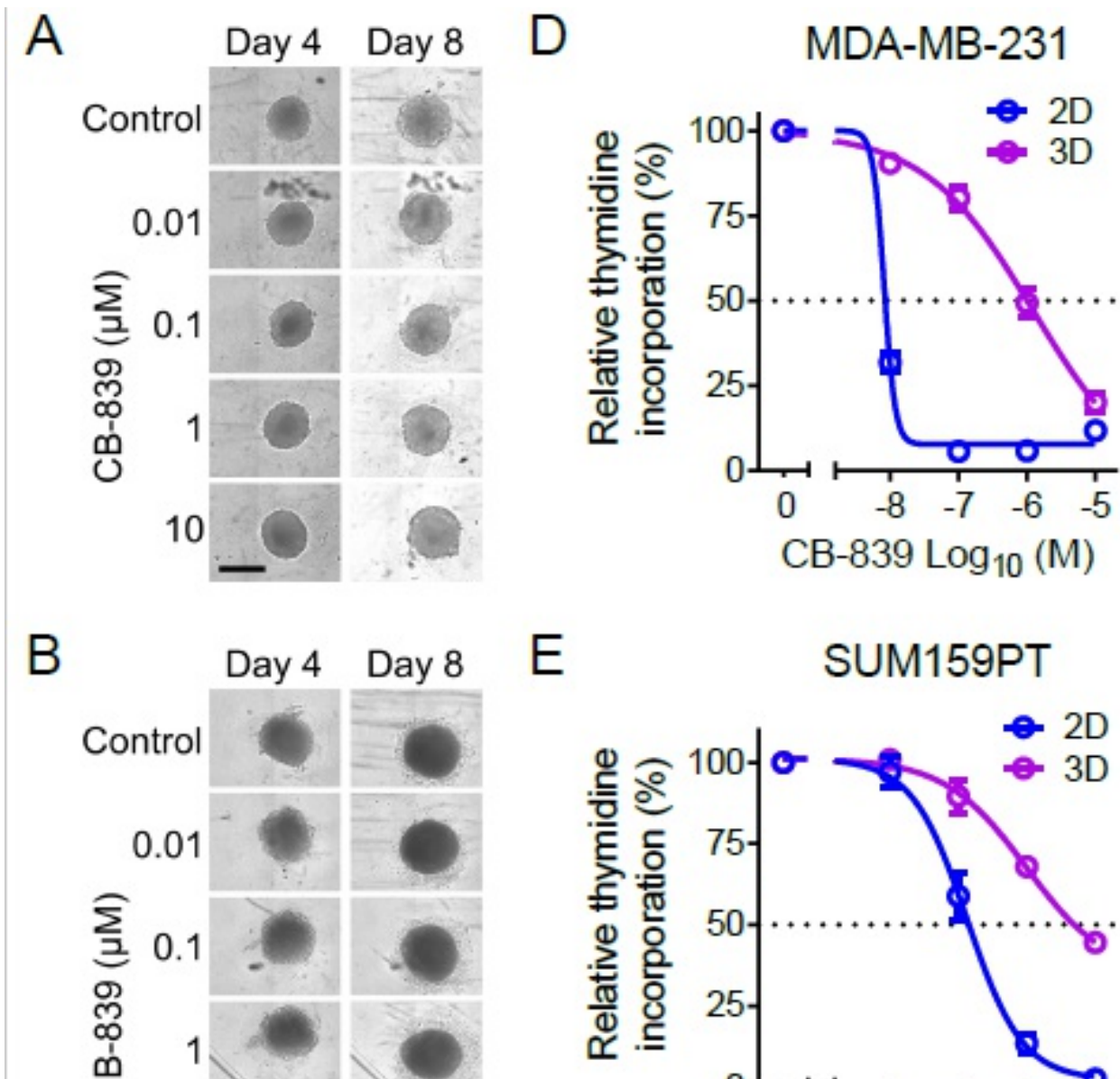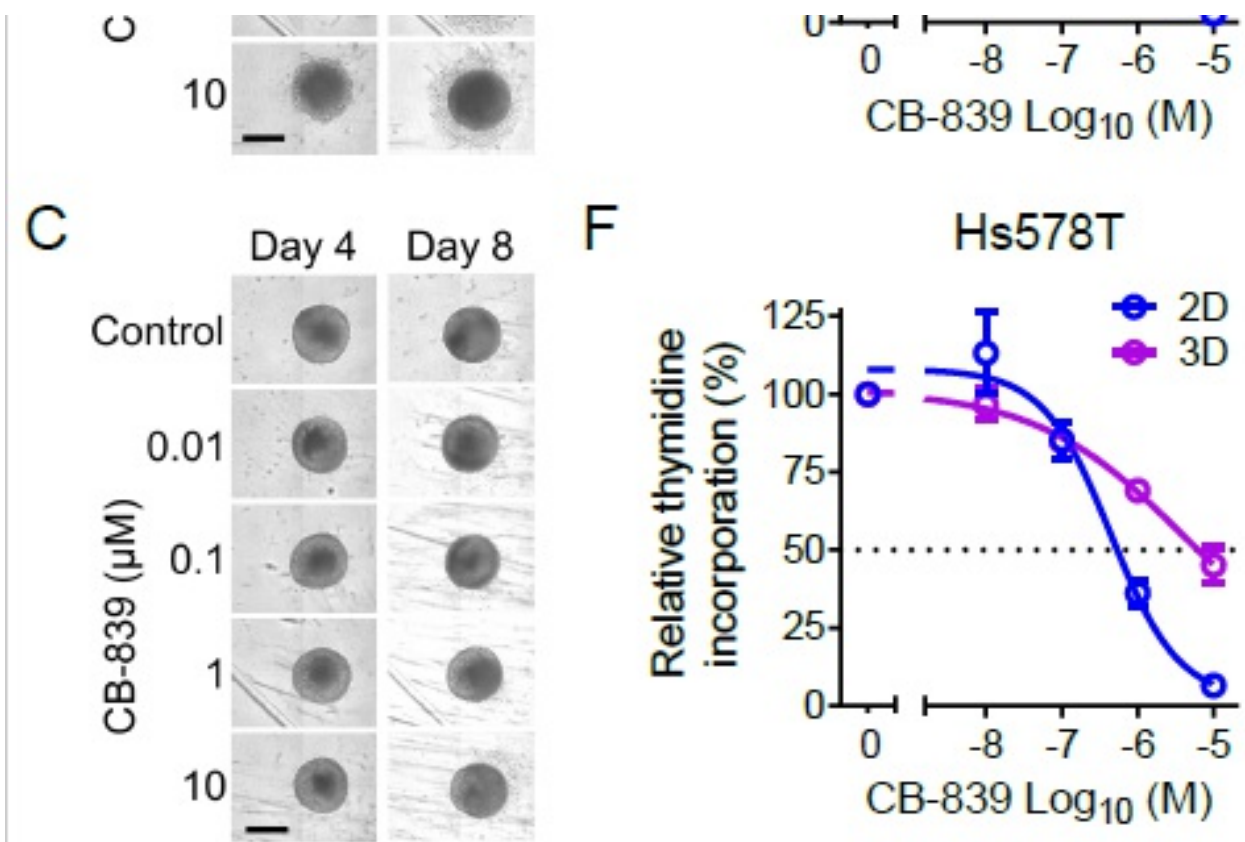
Figure 4

Activity of CB-839 in 3D spheroid versus 2D monolayer cell cultures. Images of MDA- MB-231 (A), SUM159PT (B) and Hs578T (C) 3D spheroid cell cultures captured on day 4 and 8 (scale bar = 0.5 mm). Thymidine incorporation in MDA-MB-231 (D), SUM159PT (E) and Hs578T (F) 3D spheroid cultures and 2D monolayer cultures following 4 days treatment with CB-839 (mean 555 ± SEM, n = 4-6).
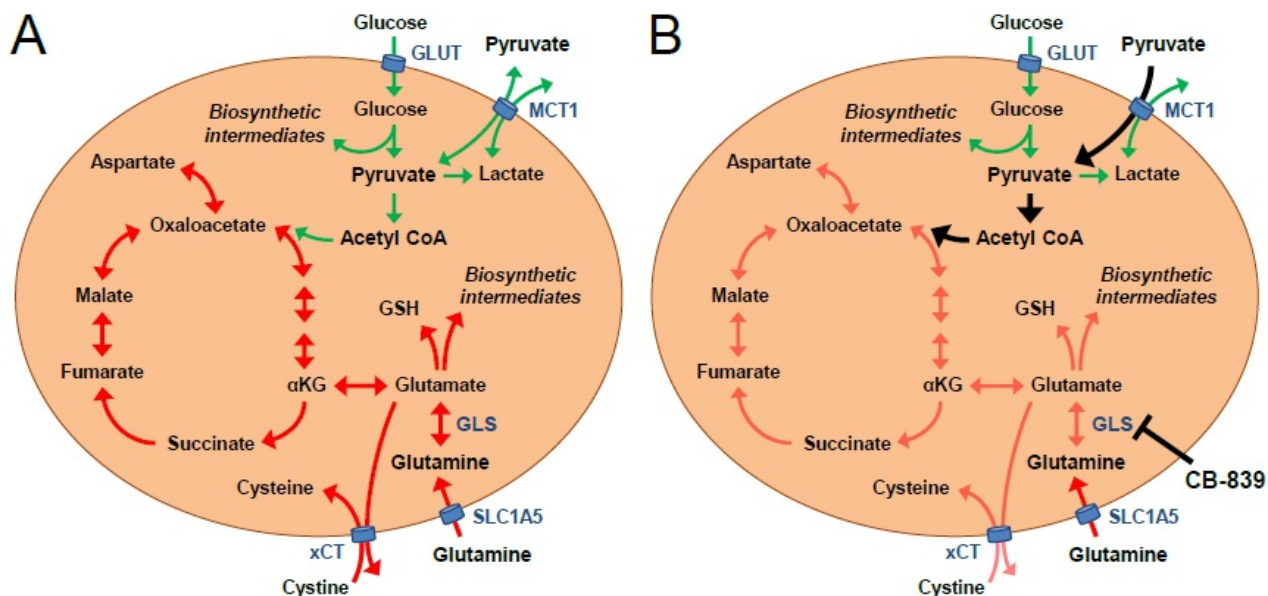
Figure 5

A Schematic highlighting metabolic preferences employed by many TNBC cells (glutamine metabolism and related processes in red arrows, glucose metabolism and related processes in green arrows). B Schematic demonstrating suppression of glutamine metabolism following glutaminase inhibition by CB-839. Extracellular pyruvate either from systemic circulation or paracrine supply can be transported into these cells to replenish TCA cycle intermediates and decrease the activity of glutaminase inhibitor. Abbreviations: GLS = glutaminase, GLUT = glucose transporter, GSH = reduced glutathione, MCT1 = monocarboxylate transporter 1, SLC1A5 = glutamine transporter, xCT= glutamate/cystine antiporter.