

Therapeutic targeting of glutaminolysis and mitochondrial TCA cycle in head and neck cancer

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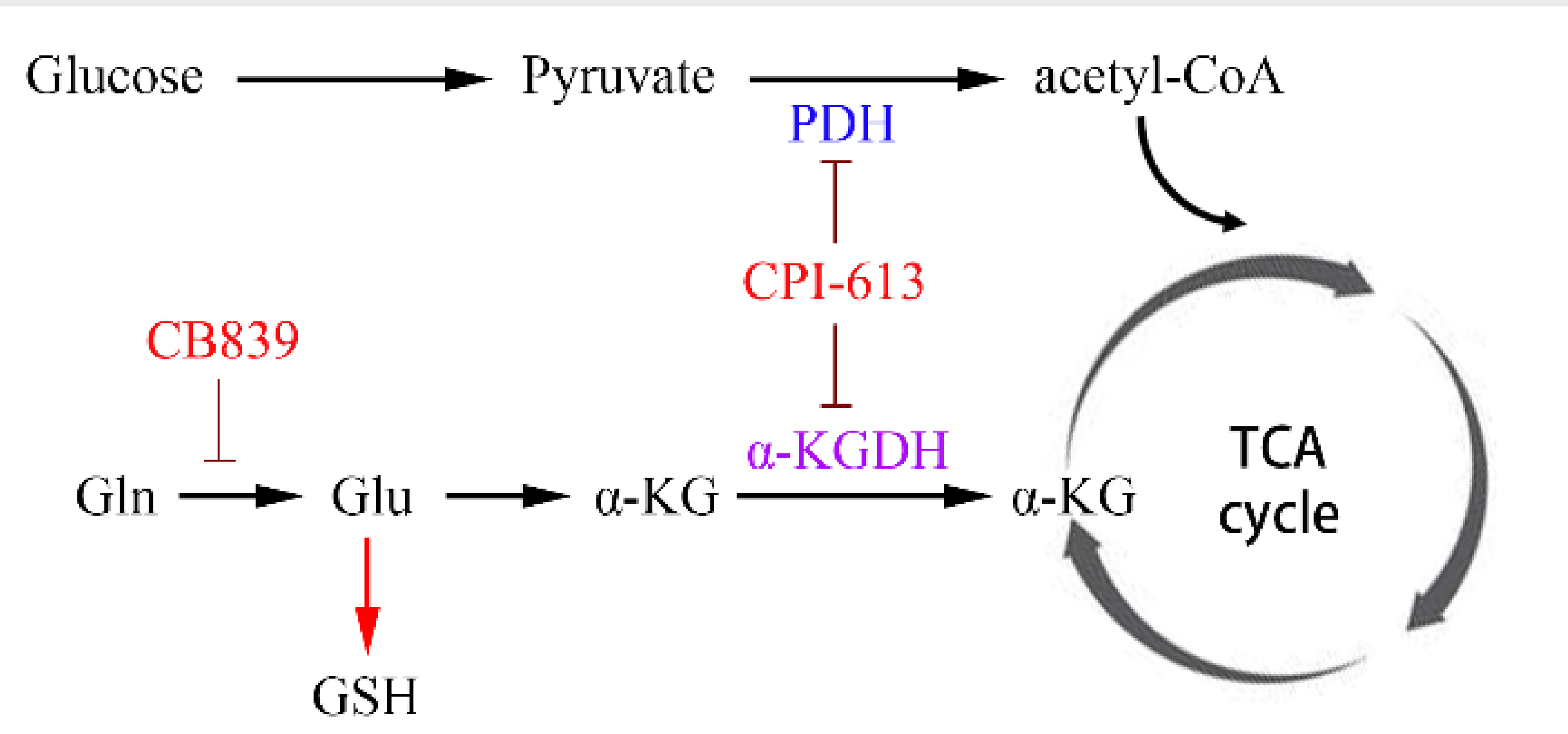
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BACKGROUND: Glutaminolysis has been established as a hallmark of cancer metabolism, which plays a compensatory role for cell survival upon alternative energy metabolism. CPI-613 (devimistat) is an effective pyruvate dehydrogenase (PDH) inhibitor that interrupts mitochondrial TCA cycle to impair energy metabolism in cancer cells. It remains unknown whether co-inhibiting glutaminolysis and energy metabolism could achieve a better therapeutic outcomes in head and neck cancer.

Methods: Gene knockdown and knockout cells were generated using lentiviral-mediated shRNAs. Alterations in cancer metabolism were determined by Glucose-Glo™ Assay Kit and Lactate-Glo™ Assay Kit. The molecular changes under different conditions were examined by Western blotting. Drug combination index (CI) values were calculated using the software ComboSyn.

Summary of Key Findings

- 1) CPI-613 resulted in alterations in head and neck cancer cell metabolism by rendering the cells with elevated glutaminase1 (GLS1) expression, leading to an increase in glutamine-dependent cell survival.
- 2) Pharmacological blockade of the glutaminolysis pathway by GLS1 inhibitor CB-839 not only decreased cancer cell viability dependent on glutaminolysis, but also improved the therapeutic effectiveness of CPI-613 in cell culture and animal models.
- 3) Co-inhibiting glutaminolysis and energy metabolism can achieve synergistic outcomes in head and neck cancer.



Results

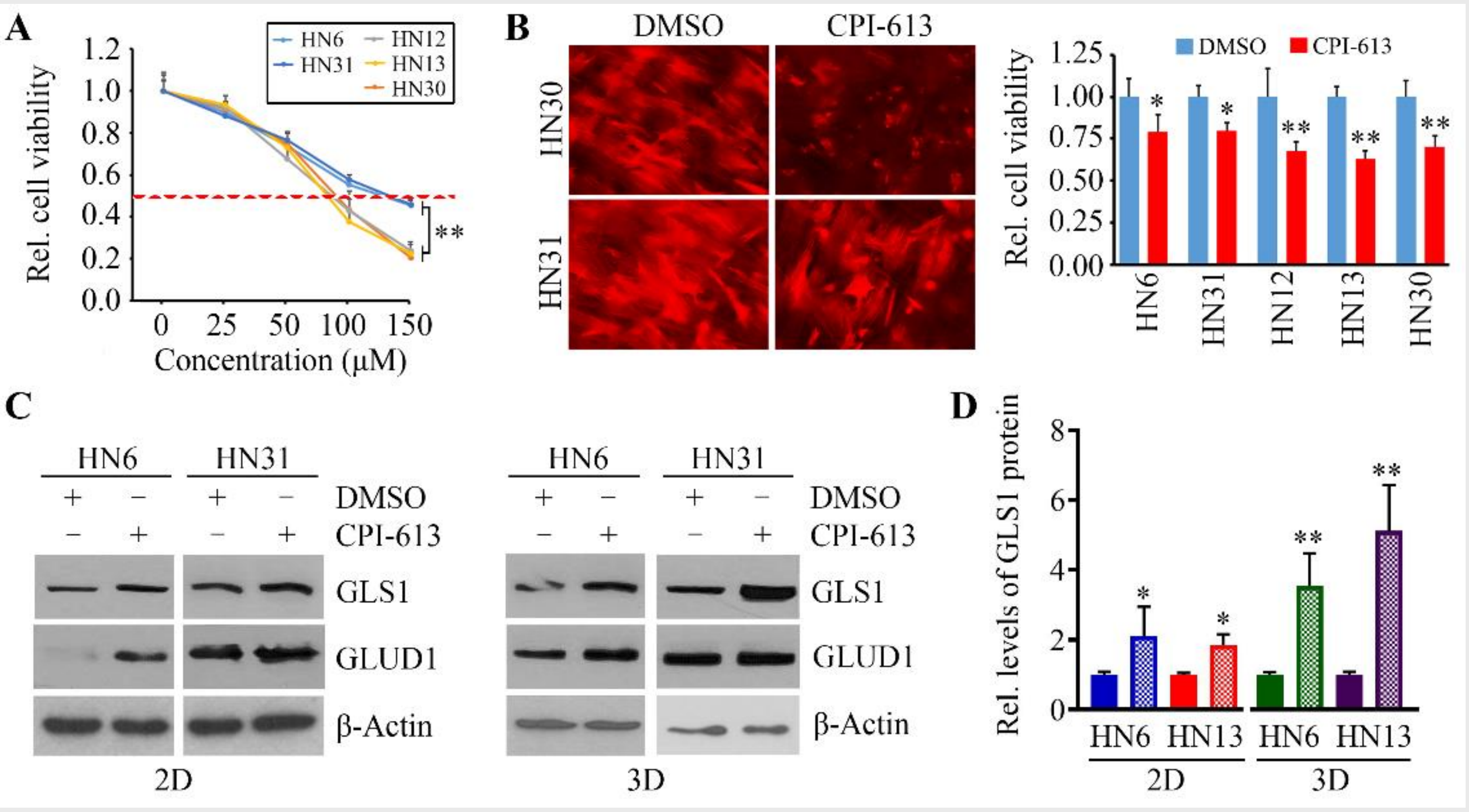


Figure 1. CPI-613 induces the upregulation of GLS1 in HNSCC cells. (A) The effect of CPI-613 on HNSCC cell viability in 2D culture plates after a three-day treatment. (B) The effect of CPI-613 on HNSCC cell viability in the SeedEZ 3D scaffold after a fourteen-day treatment. Representative images from HN30 and HN31 cells and quantitative data from three independent experiments are shown in the left and right panels, respectively. (C, D) The effect of CPI-613 on the protein levels of glycolytic and glutamine metabolism-related enzymes. Cell lysates collected from 2D culture dish and SeedEZ 3D scaffold were subjected to this analysis. Representative Western blotting images and quantitative data from three independent experiments are shown in (C) and (D), respectively. *, $p < 0.05$; **, $p < 0.01$.

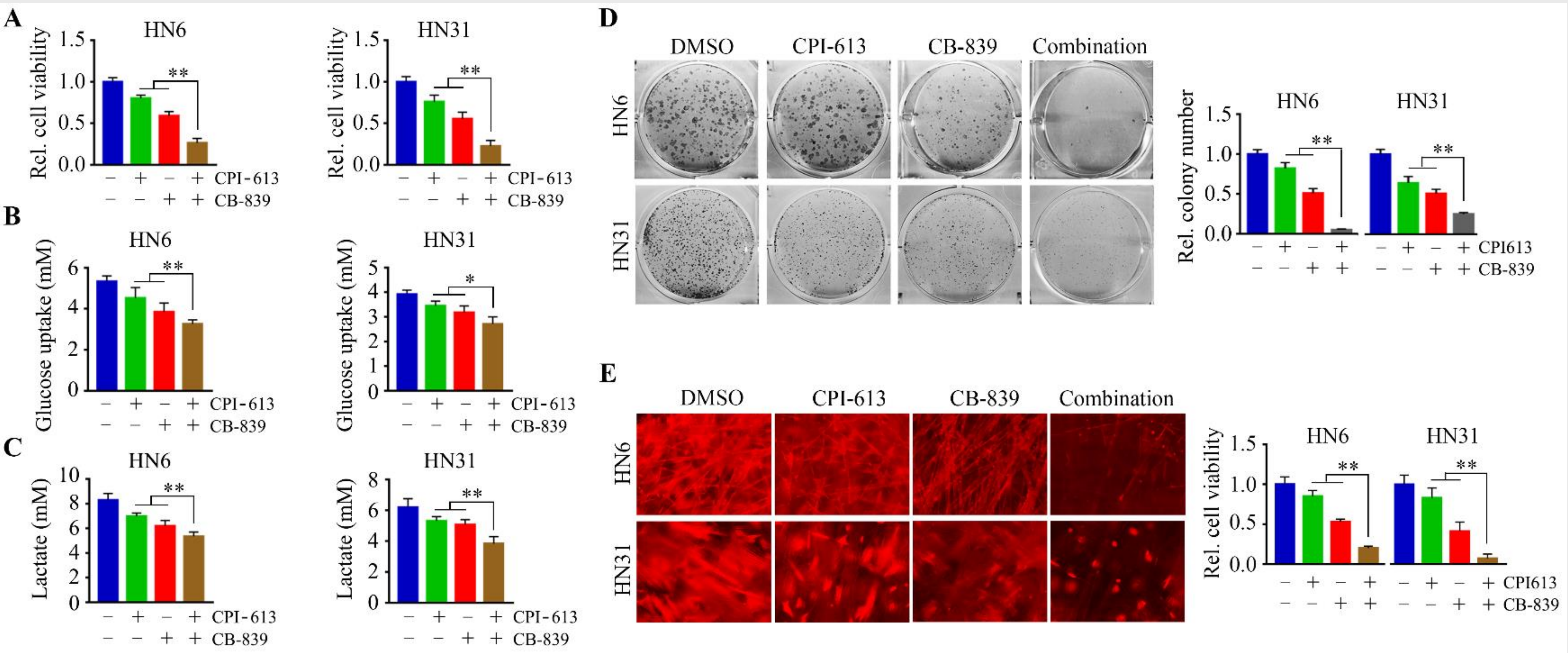


Figure 2. Inhibiting GLS1 signaling by CB-839 greatly strengthens the cytotoxic effect of CPI-613 in HNSCC cells. (A, B, C) The effect of CB-839 combined with CPI-613 on cell proliferation (A), glucose uptake (B), and lactate release (C) in HNSCC cells. (D) The effect of CB-839 combined with CPI-613 on cell colony formation in HNSCC cells. (E) The effect of CB-839 combined with CPI-613 on cell viability in the SeedEZ 3D scaffold determined on Day 14 after treatment. Representative images from HN6 and HN31 cells and quantitative data from three independent experiments are shown in the left and right panels, respectively. *, $p < 0.05$; **, $p < 0.01$.