β-HB treatment reverses sorafenib resistance through shifting glycolysis-lactate metabolism in HCC

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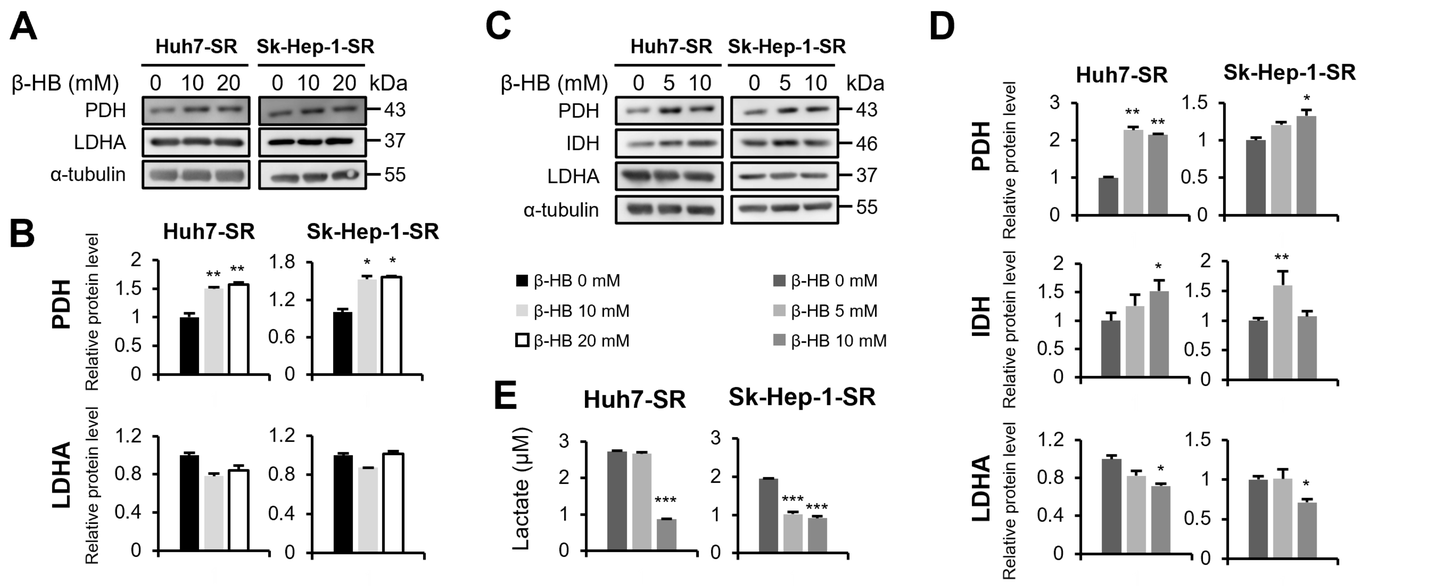
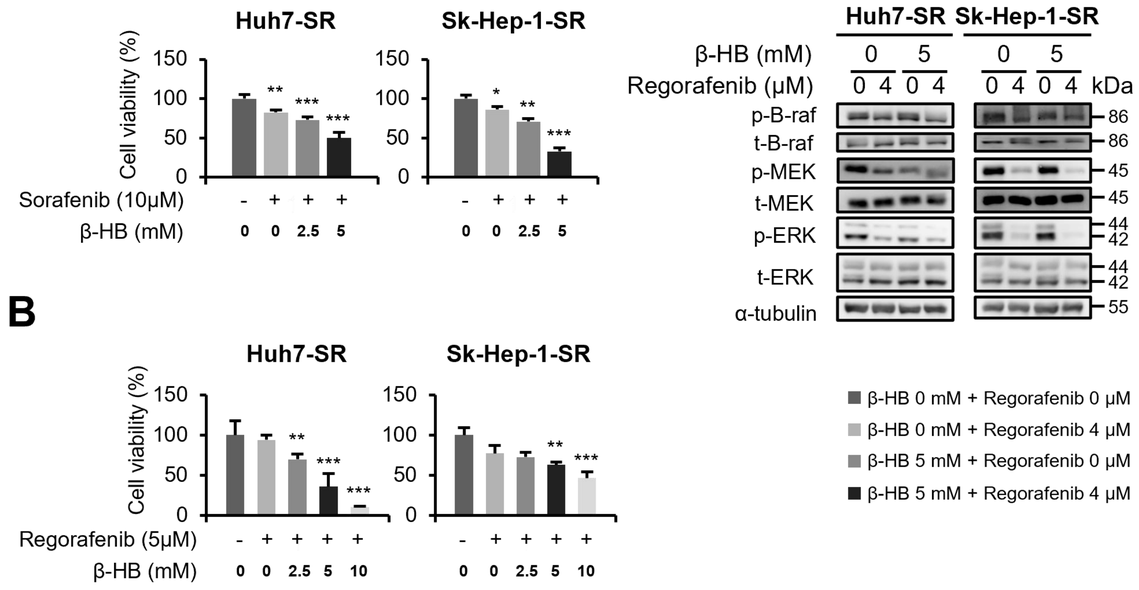
Abstract:
Hepatocellular carcinoma (HCC) is the most common primary malignant tumor in the world. Although sorafenib has been approved for patients with advanced HCC, long-term treatment often results in acquired resistance. Recently, glycolysis-mediated lactate production was reported to contribute to drug resistance and disturb the HCC treatment efficacy. This study investigated the effects of ketone body treatment in altering the metabolic shift in sorafenib-resistant HCC cells. Herein, we treated the ketone body, D-β-Hydroxybutyrate (β-HB), in two sorafenib-resistant HCC cells, and found that β-HB treatment enhanced the expression of pyruvate dehydrogenase (PDH) and decreased lactate dehydrogenase (LDH) and lactate production in sorafenib-resistant (SR) Huh7 and Sk-Hep-1 cells. Additionally, β-HB combined with sorafenib or regorafenib promoted the anti-proliferative and anti-migratory abilities of sorafenib-resistant HCC cells by inhibiting the B-raf/mitogen-activated protein kinase (MAPK) pathway and mesenchymal N-cadherin-vimentin axis. In conclusion, this study revealed that β-HB may serve as another energy source that downregulating lactate production and reverse sorafenib resistance by inducing a glycolytic shift, and it was found to possess a synergetic ability with second-line drug treatment in sorafenib-resistant HCC cells.

1. β-HB treatment reverses the glycolysis-related sorafenib resistance in Huh7 and Sk-Hep-1 cells.

β-HB treatment increased PDH protein expression levels in Huh7-SR and Sk-Hep-1-SR cells, while no significant change in LDHA levels. Furthermore, we found that cultured with 1% FBS medium promoted the enhancement of PDH and isocitrate dehydrogenase (IDH) expressions, which is the rate-limiting enzyme in the TCA cycle, in Huh7-SR and Sk-Hep-1-SR. We further detected SR-induced lactate production was reversed by β-HB treatment through dose-dependent decreases in lactate levels were observed in β-HB-treated Huh7-SR and Sk-Hep-1-SR cells. These data implied that β-HB treatment may possess the potential to transform energetic metabolism through attenuating glycolytic and lactate production in sorafenib-resistant HCC cells.



2. β-HB treatment reverses sorafenib resistance and enhances the regorafenib sensitivity of HCC-SR cells.



Different doses of β-HB with 10 μM sorafenib were used to treat Huh7-SR and Sk-Hep-1-SR cells. The cell viability significantly decreased in a dose-dependent manner under β-HB treatment. Furthermore, HCC-SR cells were treated with 5 μM regorafenib, a second-line drug for sorafenib-resistant HCC, along with β-HB, which was found significantly enhanced regorafenib cytotoxicity in HCC-SR cells. We further investigated the mechanisms associated with β-HB in enhancing regorafenib sensitivity in HCC-SR cells. The phosphorylation B-raf, MEK, and ERK notably declined after regorafenib treatment, and β-HB and regorafenib co-treatment markedly decreased the activation of B-raf, MEK, and ERK. Hence, we inferred that β-HB treatment enhanced sorafenib and regorafenib sensitivity through inhibiting the B-raf/MAPK pathway in HCC-SR cells.

3. β-HB treatment improved the cytotoxicity effects of regorafenib through inhibiting the migratory ability and EMT in sorafenib-resistant HCC cells.

We conducted a wound-healing assay to evaluate whether β-HB treatment improved the efficacy of the second-line drug, regorafenib, in sorafenib-resistant HCC cells. Treatment with 4 μM regorafenib slightly inhibited the migration of sorafenib-resistant HCC cells; however, β-HB co-treatment significantly enhanced the anti-migratory ability in both Huh7-SR and Sk-Hep-1-SR cells. Although protein levels of the epithelial-related markers, β-catenin and zona occludens (ZO)-1, exhibited no significant differences in β-HB co-treated HCC-SR cells compared to non-β-HB cotreated groups, expressions of the mesenchymal markers, vimentin and N-cadherin, decreased in cells treated with the combination of β-HB and regorafenib. These findings implied that β-HB treatment could enhance regorafenib sensitivity through regulating the EMT-mediated migratory ability of HCC-SR cells.