Targeting glutamine metabolism in colorectal cancers with PIK3CA mutations

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Abstract

Glutamine addiction is a major metabolic reprogramming event that occurs in cancer cells. Many tumors exhibit oncogene-dependent addiction to glutamine. PIK3CA, which encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase α, is the most frequently mutated oncogene in human cancers. However, whether PIK3CA mutations reprogram cancer metabolism is an important unaddressed question. Here we show that oncogenic PIK3CA mutations render colorectal cancers (CRCs) more dependent on glutamine to growth. As a metabolite, glutamine is first converted glutamate by glutaminase (GLS) and then to α-ketoglutarate (α-KG) by either a transaminase or a glutamate dehydrogenase. Calithera Biosciences recently developed a potent GLS inhibitor called CB-839, which is currently in phase I clinical trials in cancer patients. We demonstrated that CB-839 inhibits xenograft growth of CRCs with PIK3CA mutations, but not CRCs with WT PIK3CA. Remarkably, combination of CB-839 with 5-FU induces xenograft tumors regression of CRC with PIK3CA mutations, suggesting that this combinational therapy may be an effective approach to treat CRC patients whose tumors harbor PIK3CA mutations.

Mechanistically, mutant p110α up-regulates gene expression of glutamate pyruvate transaminase 2 (GPT2) in CRC cells, thereby facilitate conversion of glutamate to α-KG to replenish the tricarboxylic acid (TCA) cycle to generate ATP. Moreover, aminooxyacetate, which inhibits enzymatic activity of transaminases including GPT2, suppresses xenograft tumor growth of CRCs with PIK3CA mutations, but not CRCs with WT PIK3CA. Mechanistically, mutant p110α up-regulates the transcription of GPT2 through an AKT-independent PDK1-RSK2-ATF4 signaling axis. Our data establish oncogenic PIK3CA mutations as a cause of glutamine addiction in CRCs and that targeting glutamine metabolism may provide a novel precision therapy to treat CRCs with PIK3CA mutations.

Introduction

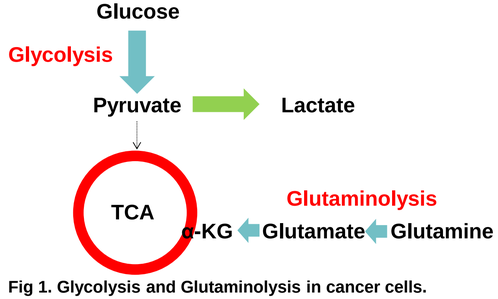

Fig 1. Glycolysis and Glutaminolysis in cancer cells.
α-KG: α-ketoglutarate

Results

Glutamine deprivation induces more apoptosis in mutant PIK3CA cell lines

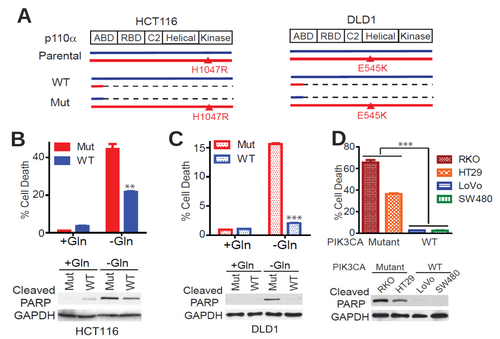

Fig 2. (A) Allele configuration of colorectal cancer lines with either the PIK3CA WT or mutant allele knocked out. **(B & C)** Glutamine deprivation induces more apoptosis in HCT116 (B) and DLD1 (C) PIK3CA mutant clones. **(D)** Glutamine deprivation induces more apoptosis in PIK3CA mutant colon cancer cell lines.

Targeting glutamate pyruvate transaminase 2 (GPT2) effectively inhibits growth of PIK3CA mutant tumors

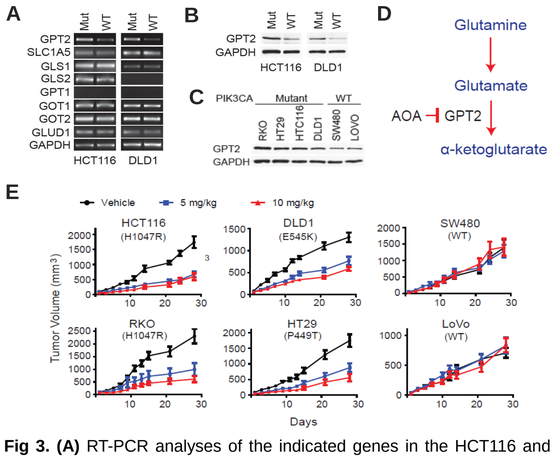

Fig 3. (A) RT-PCR analyses of the indicated genes in the HCT116 and DLD1 CRC clones. **(B & C)** Western blot analyses of GPT2 in indicated cell lines. **(D)** Aminooxyacetate (AOA) inhibits GPT2 activity. **(E)** AOA inhibits growth of xenografts formed by PIK3CA mutant cell lines.

Results

Combination of CB-839 and 5-Fu shrinks PIK3CA mutant colon cancers in xenograft models

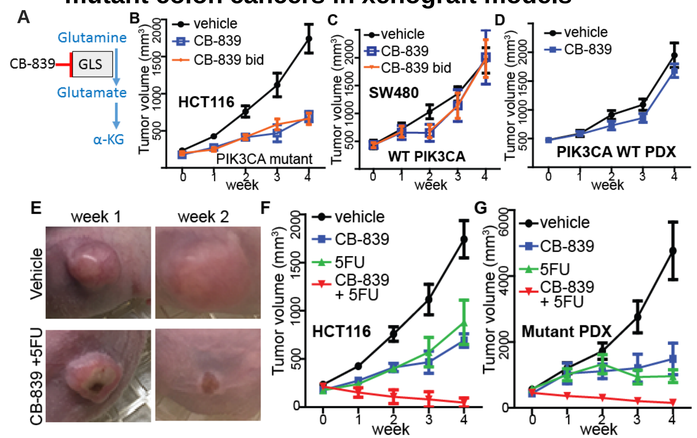

Fig 4. (A) CB-839 is a potent glutaminase (GLS) inhibitor. **(B)** CB-839 inhibits growth of PIK3CA mutant xenograft. **(C & D)** CB-839 does not inhibit xenograft growth of WT PIK3CA tumors. **(E to G)** Combination of CB-839 (200m/kg) with 5-FU (30 mg/kg) shrinks PIK3CA mutant CRCs. Representative images of tumors treated with the drugs (E). Growth curves of tumors treated with the drugs: HCT116 xenografts (F); CRC patient-derived xenograft (PDX) (G).

More α-ketoglutarates are produced in PIK3CA mutant cells

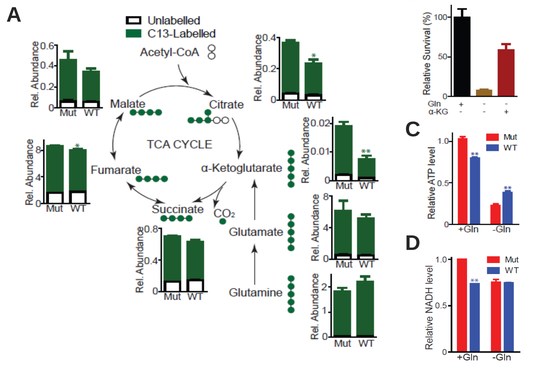

Fig 5. (A) $^{13}C_5$-Glutamine labelled TCA cycle intermediates profile in HCT116 WT and mutant (mut) clones. **(B)** α-ketoglutarate rescues cell death induced by glutamine deprivation. **(C & D)** Relative ATP and NADH level in HCT116 WT and Mut clones.

Results

Mutant p110α up-regulates transcription of GPT2 through PI3K-PDK1-RSK2-ATF4 axis

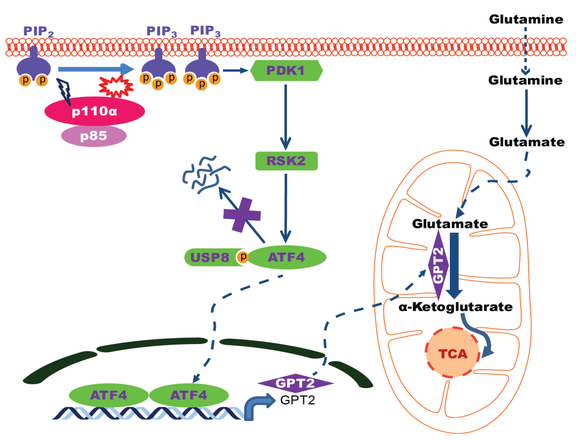

Fig 5. A model of PIK3CA/p110α mutations reprograming Glutamine metabolism.

Summary

- **PIK3CA mutant CRCs are addicted to glutamine.**
- **Combination of CB-839 with 5-FU shrinks PIK3CA mutant xenograft CRC tumors.**
- **PIK3CA mutant CRCs are sensitive to a GPT2 inhibitor.**
- **Mutant p110α regulates GPT2 through an AKT-independent PDK1-RSK2-ATF4 axis.**

Acknowledgements

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