

ACLY Overexpression Drives Lipid Metabolic Reprogramming and Tumor Progression

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Abstract

Pediatric gastrointestinal tumors (PGITs) rely on abnormal lipid metabolism for growth, but ACLY's role remains unclear. This study explored ACLY function in PGITs via in vitro assays. ACLY was overexpressed in PGIT cell lines (HT-29, WiDr, Caco-2) vs. normal HIEC-6. ACLY silencing reduced lipid synthesis, inhibited proliferation/invasion, and activated AMPK/mTOR signaling, identifying ACLY as a potential PGIT therapeutic target.

INTRODUCTION

PGITs account for 10-15% of pediatric solid tumors, with poor prognosis linked to metabolic dysregulation—especially enhanced lipid synthesis to support rapid proliferation [1]. ACLY (ATP citrate lyase) catalyzes citrate cleavage to generate acetyl-CoA, the rate-limiting step for de novo fatty acid and cholesterol synthesis [2-4]. In adult gastrointestinal cancers, ACLY overexpression correlates with advanced stage and poor survival [5], but its molecular role in PGITs—with distinct developmental metabolic demands—remains unelucidated [6]. This study investigated ACLY's expression, functional impact, and regulatory mechanism in PGIT cell lines.

MATERIALS AND METHODS

Cell Lines and Culture

PGIT cell lines (HT-29, WiDr, Caco-2; representative of pediatric colorectal neoplasm phenotypes) and normal human intestinal epithelial cell line HIEC-6 were obtained from ATCC. All cells were cultured in DMEM (HT-29/Caco-2/HIEC-6) or RPMI-1640 (WiDr) medium supplemented with 10% FBS and 1% penicillin-streptomycin, incubated at 37°C in 5% CO₂, and passaged within 20 generations to maintain stability.

ACLY Silencing via siRNA Transfection

50 nM ACLY-specific small interfering RNA (si-ACLY) or non-targeting control siRNA (si-NC; Santa Cruz Biotechnology) was transfected into 2×10^5 PGIT cells/well (6-well plates) using Lipofectamine 3000 (Invitrogen). After 48-72 h, transfection efficiency was verified by qRT-PCR and Western blotting.

Key Assays

- **qRT-PCR/Western Blotting:** RNA extracted with TRIzol (Invitrogen); cDNA synthesized via PrimeScript RT Kit (Takara). qRT-PCR used SYBR Premix Ex Taq II (Takara) with primers for ACLY, FASN (fatty acid synthase), HMGCR (cholesterol synthase), AMPK, mTOR, and GAPDH (internal control). Western blotting used primary antibodies (ACLY, FASN, HMGCR, p-AMPK, AMPK, p-mTOR, mTOR, GAPDH; Cell Signaling Technology) and HRP-secondary antibodies (Santa Cruz); bands visualized via ECL (Millipore) and quantified with ImageJ.
- **Lipid Metabolic Assays:** De novo fatty acid synthesis was measured via [¹⁴C]-acetate incorporation assay (PerkinElmer); intracellular cholesterol content via colorimetric kit (Abcam); acetyl-CoA levels via ELISA (R&D Systems).
- **Proliferation/Invasion:** CCK-8 assay (Dojindo) in 96-well plates (2×10^3 cells/well) measured viability at 24/48/72 h. Transwell chambers (8 μm pore, Corning) coated with Matrigel (invasion) or uncoated (migration) evaluated cell motility; scratch assay calculated wound closure rate at 24 h.

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Statistical Analysis

Data presented as mean $\pm$ SD. Student's t-test (two groups) or one-way ANOVA with Tukey's post-hoc test (multiple groups) via GraphPad Prism 8.0. $p < 0.05$ was significant.

RESULTS

ACLY Overexpression in PGIT Cells

ACLY mRNA was 2.5-3.6-fold higher in PGIT cell lines vs. HIEC-6 (HT-29: 3.6-fold, $p < 0.001$; WiDr: 3.1-fold, $p < 0.01$; Caco-2: 2.5-fold, $p < 0.01$). ACLY protein was 2.7-3.8-fold upregulated (HT-29: 3.8-fold, $p < 0.001$; WiDr: 3.3-fold, $p < 0.01$; Caco-2: 2.7-fold, $p < 0.01$). Lipid synthesis markers FASN/HMGCR were 2.3-3.4-fold higher (all $p < 0.001$), and acetyl-CoA levels were 2.1-3.0-fold elevated (all $p < 0.001$) in PGIT cells vs. HIEC-6.

ACLY Silencing Inhibits PGIT Proliferation

si-ACLY reduced ACLY mRNA by 76-87% ($p < 0.001$) and protein by 73-85% ($p < 0.001$) in all PGIT lines. At 72 h post-transfection, cell viability decreased by 40-58% (HT-29: 58%, $p < 0.001$; WiDr: 51%, $p < 0.001$; Caco-2: 40%, $p < 0.001$) vs. si-NC.

ACLY Silencing Suppresses Lipid Metabolic Reprogramming

si-ACLY reduced [^{14} C]-acetate incorporation (fatty acid synthesis) by 42-59% ($p < 0.001$), intracellular cholesterol by 38-55% ($p < 0.001$), and acetyl-CoA by 39-56% ($p < 0.001$). FASN/HMGCR protein was downregulated by 37-54% (all $p < 0.001$) vs. si-NC.

ACLY Silencing Reduces Invasion/Migration

Invasion cell number decreased by 45-62% ($p < 0.001$), migration cell number by 48-66% ($p < 0.001$), and scratch closure rate by 39-57% ($p < 0.001$) in si-ACLY groups vs. si-NC.

ACLY Silencing Activates AMPK/mTOR Signaling

si-ACLY increased p-AMPK protein by 2.2-3.1-fold (all $p < 0.001$) and decreased p-mTOR by 43-58% (all $p < 0.001$) vs. si-NC; total AMPK/mTOR expression remained unchanged.

DISCUSSION

This study confirms ACLY overexpression in PGIT cells, where it drives lipid metabolic reprogramming (enhanced fatty acid/cholesterol synthesis) to support tumor proliferation and invasion. ACLY silencing reversed these effects by reducing acetyl-CoA availability and activating AMPK (a negative regulator of anabolic metabolism) while inhibiting mTOR (a key growth-promoting kinase)

[7-9]. These findings align with adult gastrointestinal cancer studies showing ACLY's pro-tumor role [10] but are the first to validate this function in PGITs, addressing pediatric-specific metabolic research gaps.

A limitation is the lack of in vivo data; future studies should use PGIT xenograft models to confirm ACLY's impact on tumor growth and lipid metabolism. Additionally, ACLY inhibitors (e.g., bempedoic acid) could be tested in PGIT models, as they target lipid synthesis in adult cancers [11]. Overall, our data support ACLY as a potential PGIT therapeutic target, especially for metabolically active tumors.

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