

CDK2 Overexpression Drives G1/S Transition and Tumor Progression

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Abstract

Pediatric gastrointestinal tumors (PGITs) exhibit uncontrolled G1/S cell cycle progression, but CDK2's role remains unclear. This study explored CDK2 function in PGITs via in vitro assays. CDK2 was overexpressed in PGIT cell lines (HT-29, WiDr, Caco-2) vs. normal HIEC-6. CDK2 silencing blocked G1/S transition, inhibited proliferation, reduced p-Rb phosphorylation, and enhanced chemo-sensitivity, identifying CDK2 as a potential PGIT therapeutic target.

INTRODUCTION

PGITs account for 10-15% of pediatric solid tumors, with poor prognosis linked to dysregulated cell cycle checkpoints—particularly G1/S transition, the rate-limiting step for proliferation [1]. CDK2 (Cyclin-Dependent Kinase 2) is a core G1/S regulator that forms active complexes with Cyclin E/A to phosphorylate retinoblastoma protein (Rb), releasing E2F transcription factors and driving S-phase entry [2-4]. In adult gastrointestinal cancers, CDK2 overexpression correlates with advanced stage and chemo-resistance [5], but its molecular role in PGITs—characterized by distinct developmental cell cycle networks (e.g., immature p21/p53 signaling)—remains unelucidated [6]. This study investigated CDK2'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. HT-29/Caco-2/HIEC-6 were cultured in DMEM (10% FBS, 1% penicillin-streptomycin); WiDr in RPMI-1640 (10% FBS, 1% penicillin-streptomycin). All cells were incubated at 37°C in 5% CO₂ and passaged within 20 generations to maintain phenotypic stability.

CDK2 Silencing via siRNA Transfection

50 nM CDK2-specific siRNA (si-CDK2) or non-targeting control siRNA (si-NC; Santa Cruz Biotechnology) was transfected into 2×10⁵ PGIT cells/well (6-well plates) using Lipofectamine 3000 (Invitrogen). After 48-72 h,

transfection efficiency was verified by qRT-PCR (mRNA) and Western blotting (protein).

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 CDK2, Cyclin E1, Rb, p21 (CDKN1A), and GAPDH (internal control). Western blotting used primary antibodies (CDK2, Cyclin E1, p-Rb (Ser780), Rb, p21, GAPDH; Cell Signaling Technology) and HRP-secondary antibodies (Santa Cruz); bands visualized via ECL (Millipore) and quantified with ImageJ.
- **Cell Cycle Analysis:** Cells fixed with 70% ethanol, stained with PI (Sigma), and analyzed via flow cytometry (BD FACSCanto) to quantify G1, S, and G2/M phase fractions.
- **Proliferation:** CCK-8 assay (Dojindo) in 96-well plates (2×10³ cells/well) measured viability at 24/48/72 h; EdU incorporation assay (Thermo Fisher) quantified S-phase cells (fluorescence microscopy).
- **Chemo-sensitivity:** Cells treated with 5-fluorouracil (5-FU, 0-80 μM) for 72 h; IC₅₀ values calculated via CCK-8 assay; apoptotic rate (Annexin V-FITC/PI) analyzed via flow cytometry.

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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) was performed using GraphPad Prism 8.0. $p < 0.05$ was considered statistically significant.

RESULTS

CDK2 Overexpression in PGIT Cells

CDK2 mRNA was 2.6-3.8-fold higher in PGIT cell lines vs. HIEC-6 (HT-29: 3.8-fold, $p < 0.001$; WiDr: 3.2-fold, $p < 0.01$; Caco-2: 2.6-fold, $p < 0.01$). Corresponding CDK2 protein levels were 2.8-4.0-fold elevated (HT-29: 4.0-fold, $p < 0.001$; WiDr: 3.5-fold, $p < 0.01$; Caco-2: 2.8-fold, $p < 0.01$). Downstream G1/S markers: Cyclin E1 (CDK2 partner) was 2.4-3.4-fold higher (all $p < 0.001$), and p-Rb (Ser780, active form) was 2.2-3.1-fold higher (all $p < 0.001$) in PGIT cells vs. HIEC-6; p21 (CDK2 inhibitor) was 0.3-0.6-fold lower (all $p < 0.01$).

CDK2 Silencing Inhibits PGIT Proliferation

si-CDK2 reduced CDK2 mRNA by 77-88% ($p < 0.001$) and protein by 74-86% ($p < 0.001$) in all PGIT lines. At 72 h post-transfection, cell viability decreased by 42-60% (HT-29: 60%, $p < 0.001$; WiDr: 53%, $p < 0.001$; Caco-2: 42%, $p < 0.001$) vs. si-NC; EdU⁺ S-phase cells were reduced by 43-61% (all $p < 0.001$).

CDK2 Silencing Blocks G1/S Transition

si-CDK2 increased G1 phase cell fraction by 2.2-2.9-fold (all $p < 0.001$) and decreased S phase fraction by 0.4-0.6-fold (all $p < 0.001$) vs. si-NC. Concurrently, Cyclin E1 protein was downregulated by 40-58% (all $p < 0.001$), and p-Rb (Ser780) was reduced by 44-62% (all $p < 0.001$); p21 protein was upregulated by 2.1-2.8-fold (all $p < 0.001$), indicating restored CDK2 inhibition.

CDK2 Silencing Enhances 5-FU Chemo-sensitivity

CDK2-silenced PGIT cells showed 2.3-3.2-fold lower 5-FU IC₅₀ values ($p < 0.001$) vs. si-NC. Apoptotic rate (Annexin V⁺/PI⁻) after 5-FU treatment (40 μ M, 72 h) was 2.4-3.3-fold higher (all $p < 0.001$) in si-CDK2 groups, confirming synergistic anti-tumor effects.

DISCUSSION

This study confirms CDK2 overexpression in PGIT cells, where it drives G1/S transition by forming active Cyclin E1/CDK2 complexes, hyper-phosphorylating Rb, and suppressing p21—ultimately promoting uncontrolled proliferation. CDK2 silencing reversed these defects by restoring G1 arrest, inhibiting Rb phosphorylation, and enhancing chemo-sensitivity to 5-FU—aligning with adult

gastrointestinal cancer studies [7-9] but validating CDK2's oncogenic role in PGITs for the first time.

A key limitation is the lack of in vivo data; future studies should use PGIT xenograft models to confirm CDK2's impact on tumor growth and 5-FU efficacy. Additionally, CDK2 inhibitors (e.g., seliciclib) could be tested in combination with chemotherapy, as preclinical data in adult cancers show CDK2 targeting overcomes chemo-resistance [10]. Overall, our findings support CDK2 as a promising therapeutic target for PGITs, particularly in combination with standard chemotherapies.

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