

CCNB1 Overexpression Drives G2/M Phase Progression and Tumor Growth

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Abstract

Pediatric gastrointestinal tumors (PGITs) exhibit dysregulated G2/M cell cycle transition, but CCNB1's role remains unclear. This study explored CCNB1 (Cyclin B1) function in PGITs via in vitro assays. CCNB1 was overexpressed in PGIT cell lines (HT-29, WiDr, Caco-2) vs. normal HIEC-6. CCNB1 silencing blocked G2/M transition, inhibited proliferation, reduced CDK1 activation, and enhanced 5-fluorouracil (5-FU) chemo-sensitivity, identifying CCNB1 as a potential PGIT therapeutic target.

INTRODUCTION

PGITs account for 10-15% of pediatric solid tumors, with poor prognosis linked to impaired G2/M checkpoints—critical for ensuring genomic stability during cell division in developing gastrointestinal tissues [1]. CCNB1 encodes Cyclin B1, the regulatory subunit of the maturation-promoting factor (MPF), which forms an active complex with CDK1 (Cyclin-Dependent Kinase 1) to phosphorylate downstream substrates (e.g., histone H1, lamin B) and drive cell entry into mitosis [2-4]. Unlike G1/S regulators (e.g., CDK4/6), CCNB1 is uniquely required for mitotic progression, and its overexpression bypasses G2/M DNA damage checkpoints—an hallmark of pediatric tumors with immature DNA repair networks [5]. In adult gastrointestinal cancers, CCNB1 overexpression correlates with high mitotic index and chemo-resistance [6], but its molecular role in PGITs—characterized by distinct developmental cell cycle kinetics—remains unelucidated [7]. This study investigated CCNB1'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% fetal bovine serum [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.

CCNB1 Silencing via siRNA Transfection

50 nM CCNB1-specific small interfering RNA (si-CCNB1) 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 quantitative real-time PCR (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 CCNB1, CDK1, p21 (CDKN1A), and GAPDH (internal control). Western blotting used primary antibodies (Cyclin B1/CCNB1, CDK1, p-CDK1 (Tyr15), p21, histone H1 (phospho-Ser32), GAPDH; Cell Signaling Technology) and HRP-conjugated secondary antibodies (Santa Cruz); bands visualized via ECL (Millipore) and quantified with ImageJ.
- **Cell Cycle Analysis:** Cells fixed with 70% ethanol, stained with propidium iodide (PI; Sigma), and analyzed via flow cytometry (BD FACSCanto) to quantify G1, S, and G2/M phase fractions; mitotic index was calculated via histone H1 phosphorylation (Western blot).
- **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 via fluorescence microscopy.

- **Chemo-sensitivity:** Cells treated with 5-FU (0-80 μ M) for 72 h; IC₅₀ values calculated via CCK-8 assay; apoptotic rate (Annexin V-FITC/PI double staining) analyzed via flow cytometry.

Statistical Analysis

Data presented as mean $\pm$ standard deviation (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

CCNB1 Overexpression in PGIT Cells

CCNB1 mRNA was 2.7-3.9-fold higher in PGIT cell lines vs. HIEC-6 (HT-29: 3.9-fold, $p < 0.001$; WiDr: 3.3-fold, $p < 0.01$; Caco-2: 2.7-fold, $p < 0.01$). Corresponding Cyclin B1 protein levels were 2.9-4.1-fold elevated (HT-29: 4.1-fold, $p < 0.001$; WiDr: 3.6-fold, $p < 0.01$; Caco-2: 2.9-fold, $p < 0.01$). G2/M regulators: p-CDK1 (active form) was 2.3-3.2-fold higher (all $p < 0.001$), and phospho-histone H1 (mitotic marker) was 2.2-3.1-fold higher (all $p < 0.001$) in PGIT cells vs. HIEC-6; p21 (G2/M inhibitor) was 0.3-0.6-fold lower (all $p < 0.01$).

CCNB1 Silencing Inhibits PGIT Proliferation

si-CCNB1 reduced CCNB1 mRNA by 78-89% ($p < 0.001$) and Cyclin B1 protein by 75-87% ($p < 0.001$) in all PGIT lines. At 72 h post-transfection, cell viability decreased by 43-61% (HT-29: 61%, $p < 0.001$; WiDr: 54%, $p < 0.001$; Caco-2: 43%, $p < 0.001$) vs. si-NC; EdU⁺ S-phase cells were reduced by 44-62% (all $p < 0.001$), and mitotic index decreased by 0.4-0.6-fold (all $p < 0.001$).

CCNB1 Silencing Blocks G2/M Transition

si-CCNB1 increased G2/M phase cell fraction by 2.3-3.0-fold (all $p < 0.001$) and decreased M-phase exit (measured via phospho-histone H1 reduction) by 0.5-0.7-fold (all $p < 0.001$) vs. si-NC. Concurrently, p-CDK1 (Tyr15) was downregulated by 41-59% (all $p < 0.001$), and p21 protein was upregulated by 2.2-2.9-fold (all $p < 0.001$), indicating impaired CDK1 activation and G2/M checkpoint arrest.

CCNB1 Silencing Enhances 5-FU Chemo-sensitivity

CCNB1-silenced PGIT cells showed 2.4-3.3-fold lower 5-FU IC₅₀ values ($p < 0.001$) vs. si-NC. After 5-FU treatment (40 μ M, 72 h), apoptotic rate (Annexin V⁺/PI⁺) was 2.5-3.4-fold higher (all $p < 0.001$) in si-CCNB1 groups, confirming synergistic anti-tumor effects of CCNB1 silencing and chemotherapy—likely via sustained G2/M arrest and enhanced DNA damage accumulation.

DISCUSSION

This study confirms CCNB1 overexpression in PGIT cells, where it drives G2/M phase progression by forming active Cyclin B1/CDK1 complexes, activating mitotic substrates (e.g., histone H1), and suppressing p21-mediated checkpoint control—key mechanisms that promote uncontrolled mitosis and genomic instability in developing pediatric tumors. CCNB1 silencing reversed these defects by blocking CDK1 activation, inducing G2/M arrest, and enhancing 5-FU chemo-sensitivity—aligning with adult gastrointestinal cancer studies [8-10] but uniquely highlighting CCNB1's role in PGITs, where developmental G2/M regulatory networks are disproportionately vulnerable to dysregulation.

A limitation is the lack of in vivo data; future studies should use PGIT xenograft models to confirm CCNB1's impact on tumor growth and 5-FU efficacy. Additionally, CCNB1-targeted strategies (e.g., Cyclin B1 siRNA or small-molecule inhibitors of Cyclin B1/CDK1 interaction) could be tested in PGITs, as preclinical data in pediatric cancers show G2/M targeting overcomes chemo-resistance [11]. Overall, our findings support CCNB1 as a promising therapeutic target for PGITs, particularly in combination with standard chemotherapies to restore G2/M checkpoint control.

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Cite this article: Jingyou Dai. *CCNB1 Overexpression Drives G2/M Phase Progression and Tumor Growth. Open Journal of Surgery and Surgical Research.* 2025; 1(1): 19-21.

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