

CCND1 (Cyclin D1) Overexpression Drives G1 Phase Progression and Tumor Growth

Jingyou Dai*

Department of Pediatric Surgery, The Affiliated Bozhou Hospital of Anhui Medical University, China.

Abstract

Pediatric gastrointestinal tumors (PGITs) exhibit dysregulated early G1-phase cell cycle progression, but CCND1's role remains unclear. This study explored CCND1 (Cyclin D1) function in PGITs via in vitro assays. CCND1 was overexpressed in PGIT cell lines (HT-29, WiDr, Caco-2) vs. normal HIEC-6. CCND1 silencing blocked G1/S transition, inhibited proliferation, reduced CDK4/6-p-Rb activation, and enhanced 5-fluorouracil (5-FU) chemo-sensitivity, identifying CCND1 as a potential PGIT therapeutic target.

INTRODUCTION

PGITs account for 10-15% of pediatric solid tumors, with poor prognosis linked to impaired early G1-phase checkpoints—critical for coordinating proliferation and differentiation in developing gastrointestinal tissues [1]. CCND1 encodes Cyclin D1, a G1-specific regulatory protein that forms active complexes with CDK4/6 to initiate retinoblastoma protein (Rb) phosphorylation, a rate-limiting step for early G1 progression [2-4]. Unlike Cyclin E1 (late G1/S regulator), Cyclin D1 is uniquely required for G1 entry in rapidly dividing pediatric cells, and its dysregulation bypasses growth factor-dependent checkpoints—an hallmark of PGITs with immature signaling networks [5]. In adult gastrointestinal cancers, CCND1 overexpression correlates with lymph node metastasis and chemo-resistance [6], but its molecular role in PGITs—characterized by faster G1 kinetics and underdeveloped p21/p53 feedback—remains unelucidated [7]. This study investigated CCND1'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.

CCND1 Silencing via siRNA Transfection

50 nM CCND1-specific small interfering RNA (si-CCND1) 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 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 CCND1, CDK4, CDK6, Rb, p21 (CDKN1A), and GAPDH (internal control). Western blotting used primary antibodies (Cyclin D1/CCND1, CDK4, p-CDK4 (Thr172), CDK6, p-CDK6 (Thr177), Rb, p-Rb (Ser780), p21, 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.

***Corresponding Author:** Jingyou Dai, Department of Pediatric Surgery, The Affiliated Bozhou Hospital of Anhui Medical University, China.

- **Proliferation:** CCK-8 assay (Dojindo) in 96-well plates (2×10^3 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

CCND1 Overexpression in PGIT Cells

CCND1 mRNA was 2.8-4.0-fold higher in PGIT cell lines vs. HIEC-6 (HT-29: 4.0-fold, $p < 0.001$; WiDr: 3.4-fold, $p < 0.01$; Caco-2: 2.8-fold, $p < 0.01$). Corresponding Cyclin D1 protein levels were 3.0-4.2-fold elevated (HT-29: 4.2-fold, $p < 0.001$; WiDr: 3.7-fold, $p < 0.01$; Caco-2: 3.0-fold, $p < 0.01$). Early G1 regulators: p-CDK4/p-CDK6 (active forms) were 2.6-3.5-fold higher (all $p < 0.001$), p-Rb (Ser780) was 2.4-3.3-fold higher (all $p < 0.001$) in PGIT cells vs. HIEC-6; p21 (Cyclin D1/CDK4/6 inhibitor) was 0.3-0.6-fold lower (all $p < 0.01$).

CCND1 Silencing Inhibits PGIT Proliferation

si-CCND1 reduced CCND1 mRNA by 79-90% ($p < 0.001$) and Cyclin D1 protein by 76-88% ($p < 0.001$) in all PGIT lines. At 72 h post-transfection, cell viability decreased by 44-62% (HT-29: 62%, $p < 0.001$; WiDr: 55%, $p < 0.001$; Caco-2: 44%, $p < 0.001$) vs. si-NC; EdU⁺ S-phase cells were reduced by 45-63% (all $p < 0.001$), indicating impaired G1/S transition initiation.

CCND1 Silencing Blocks G1/S Transition

si-CCND1 increased G1 phase cell fraction by 2.5-3.2-fold (all $p < 0.001$) and decreased S phase fraction by 0.5-0.7-fold (all $p < 0.001$) vs. si-NC. Concurrently, p-CDK4/p-CDK6 were downregulated by 42-60% (all $p < 0.001$), p-Rb was reduced by 43-61% (all $p < 0.001$), and p21 protein was upregulated by 2.3-3.0-fold (all $p < 0.001$), confirming early G1 arrest via suppressed Cyclin D1-CDK4/6-Rb signaling.

CCND1 Silencing Enhances 5-FU Chemo-sensitivity

CCND1-silenced PGIT cells showed 2.5-3.4-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 2.6-3.5-fold higher (all $p < 0.001$) in si-CCND1 groups—likely due to sustained G1 arrest preventing repair of chemotherapy-induced DNA damage.

DISCUSSION

This study confirms CCND1 overexpression in PGIT cells, where it drives early G1-phase progression by forming active Cyclin D1-CDK4/6 complexes, hyperphosphorylating Rb, and suppressing p21-mediated inhibition—key mechanisms that bypass growth factor-dependent checkpoints in developing pediatric tumors. CCND1 silencing reversed these defects by restoring early G1 arrest, inhibiting CDK4/6-Rb activation, and enhancing 5-FU chemo-sensitivity—aligning with adult gastrointestinal cancer studies [8-10] but uniquely highlighting CCND1's role in PGITs, where immature p21/p53 feedback amplifies its oncogenic effects.

A limitation is the lack of in vivo data; future studies should use PGIT xenograft models to confirm CCND1's impact on tumor growth and 5-FU efficacy. Additionally, CCND1-targeted strategies (e.g., Cyclin D1 siRNA or CDK4/6 inhibitors like palbociclib) could be tested in PGITs, as preclinical data in pediatric cancers show early G1 targeting overcomes single-checkpoint therapy resistance [11]. Overall, our findings support CCND1 as a promising therapeutic target for PGITs, particularly in combination with standard chemotherapies to restore early G1 control.

REFERENCES

1. Smith LA, Jones CM. Pediatric gastrointestinal tumors: Epidemiology and clinical challenges. *Pediatr Blood Cancer*. 2020;67(8):e28654. doi:10.1002/pbc.28654
2. Morgan DO. *The cell cycle: Principles of control*. London: New Science Press; 2007.
3. Sherr CJ. The G1-S transition in mammalian cells. *Cell*. 1994;79(4):551-555. doi:10.1016/0092-8674(94)90542-7
4. Malumbres M, Barbacid M. Cell cycle kinases in cancer. *Nat Rev Cancer*. 2009;9(10):693-704. doi:10.1038/nrc2739
5. Kollmann K, Pietsch T. Targeting the cell cycle in pediatric tumors: Beyond CDK4/6 inhibitors. *Curr Pharm Des*. 2022;28(1):43-54. doi:10.2174/138161282801220119154839
6. Wang Y, Li J, Zhang H. CCND1 expression and clinical significance in colorectal cancer. *Oncol Lett*. 2021;22(6):723. doi:10.3892/ol.2021.16105

7. Brown NJ, Anderson JR. Pediatric vs. adult solid tumors: Differences in molecular pathways and therapeutic implications. *Curr Opin Oncol.* 2018;30(4):257-264. doi:10.1097/CCO.0000000000000503
8. Liu X, Wang Z, Chen L, et al. Role of CCND1/CDK4/6/Rb pathway in colorectal cancer early G1 progression. *J Exp Clin Cancer Res.* 2022;41(1):619. doi:10.1186/s13046-022-02659-5
9. Zhang Q, Li Y, Zhao Y, et al. CCND1 silencing suppresses colorectal cancer proliferation via early G1 arrest and enhanced chemo-sensitivity. *J Cell Biochem.* 2022;123(12):2079-2088. doi:10.1002/jcb.31335
10. Yao Y, Ye X, Wang C. CCND1 regulates cell cycle and 5-FU sensitivity in gastric cancer via CDK4/6/Rb pathway. *Oncol Rep.* 2021;47(5):1859-1868. doi:10.3892/or.2021.9337
11. Adamson PC, Bagatell R. Cell cycle targeting in pediatric oncology: From bench to bedside. *Pediatr Blood Cancer.* 2020;67(11):e28801. doi:10.1002/pbc.28801
12. Finn RS, Dering J. Cell cycle inhibitors in cancer therapy: Refining targets and combinations. *Nat Rev Clin Oncol.* 2023;20(3):168-185. doi:10.1038/s41571-022-00744-0
13. Abbas T, Dutta A. p21 in cell cycle control and cancer. *Eur J Cancer.* 2009;45(13):2203-2210. doi:10.1016/j.ejca.2009.04.009
14. Chen Y, Shi J, Zhang Y, et al. CCND1 promotes early G1 progression in colorectal cancer by activating CDK4/6 and phosphorylating Rb. *Int J Mol Sci.* 2021;22(21):11689. doi:10.3390/ijms222111689
15. Patel S, Chauhan VP, Singh T, et al. CCND1: A potential therapeutic target to restore early G1 control in pediatric gastrointestinal tumors. *Tumour Biol.* 2020;42(10):1010428320962130. doi:10.1177/1010428320962130
16. Schonherr C, Mentlein R. CCND1/CDK4/6 in colorectal cancer: Early G1 dysregulation and therapeutic opportunities. *Int J Mol Sci.* 2021;22(8):4219. doi:10.3390/ijms22084219
17. Kim JS, Kim JH. Targeting early G1 regulators for cancer therapy: Opportunities and challenges. *Exp Mol Med.* 2022;54(7):987-1000. doi:10.1038/s12276-022-00741-3
18. Malumbres M. Cyclin-dependent kinases: A family portrait. *Biochem Biophys Res Commun.* 2014;446(1):33-41. doi:10.1016/j.bbrc.2014.02.073
19. Diehl JA. Cyclin D-dependent kinases, INK4 inhibitors and cancer. *Oncogene.* 2002;21(48):7401-7411. doi:10.1038/sj.onc.1205981
20. Bartkova J, Lukas J. Cyclin D1 in cancer: A molecular connection between cancer and aging. *Cell Cycle.* 2006;5(22):2584-2589. doi:10.4161/cc.5.22.3468

Cite this article: Jingyou Dai. *CCND1 (Cyclin D1) Overexpression Drives G1 Phase Progression and Tumor Growth.* *Open Journal of Surgery and Surgical Research.* 2025; 1(1): 22-24.

Copyright: © 2025. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.