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Cell cycle checkpoint regulation by cyclin-dependent kinase inhibitors in colorectal carcinoma

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Abstract

While Cyclin-Dependent Kinases (CDKs) drive cell cycle progression, their endogenous inhibitors notably p21 and p27 act as brakes that enforce checkpoint control. Colorectal carcinoma frequently disrupts this balance. This research examined how pharmacological CDK inhibition affects checkpoint activation and cell viability in p21-positive versus p21-null colorectal cancer cell lines. Experiments were performed at the Fynbos Institute of Biodiversity, Stellenbosch, South Africa, from June 2021 to March 2023. Three CDK inhibitors (palbociclib, ribociclib, and abemaciclib) were tested at concentrations ranging from 0.5 to 50 μM on HCT116 (p21+/+) and HCT116 p21-/- isogenic cell lines. Flow cytometry revealed that G1 arrest was the dominant response in p21-positive cells (38.7% of treated cells), while p21-null cells showed preferential G2/M arrest (31.4%). Cell viability at 48 hours was significantly lower in p21-positive cells across all three inhibitors (IC50 values 4.2-8.7 μM vs 11.3-19.6 μM in p21-null cells, $p < 0.01$). Western blot analysis confirmed that CDK4/6 inhibition triggered p21 accumulation in wild-type cells, amplifying the G1 checkpoint. These findings indicate that p21 status shapes the checkpoint response to CDK inhibitors and may influence therapeutic sensitivity in colorectal carcinoma.

Keywords: Cyclin-dependent kinase inhibitors, cell cycle checkpoints, p21, colorectal carcinoma, G1 arrest, palbociclib, flow cytometry, tumor suppression

Introduction

Cancer cells and normal cells face the same cell cycle machinery, but they handle it in opposite ways. Normal cells obey checkpoint signals that halt division when DNA is damaged or conditions are unfavorable; cancer cells override these signals to proliferate without restraint ^[1]. In colorectal carcinoma the third most common malignancy worldwide checkpoint evasion frequently involves loss or silencing of CDK inhibitor proteins, particularly p21WAF1/CIP1 and p27KIP1 ^[2].

CDKs 4 and 6 phosphorylate the retinoblastoma protein (Rb) during G1 phase, releasing E2F transcription factors that drive S-phase entry. The endogenous inhibitor p21 blocks CDK4/6 activity, enforcing the G1/S checkpoint ^[3]. When p21 is absent or non-functional, cells become dependent on alternative checkpoints particularly the G2/M checkpoint regulated by CDK1 and its inhibitor Wee1 to maintain any remaining quality control over division ^[4].

Pharmacological CDK4/6 inhibitors (palbociclib, ribociclib, abemaciclib) have transformed the treatment of hormone receptor-positive breast cancer, but their efficacy in colorectal carcinoma has been inconsistent ^[5, 6]. One explanation is that the checkpoint response to these drugs depends on the tumor's endogenous CDK inhibitor status a variable that hasn't been systematically tested in isogenic colorectal models. This research used HCT116 p21+/+ and p21-/- isogenic cell lines to directly compare how p21 status shapes the cell cycle response to CDK4/6 inhibition, with the aim of identifying whether p21 expression predicts therapeutic sensitivity ^[7, 8].

Ethical considerations

This research used established human cell lines (HCT116 and its isogenic derivative HCT116 p21-/-) obtained from the American Type Culture Collection (ATCC) and the Vogelstein laboratory collection, respectively. No primary human tissue or patient samples were used. Institutional biosafety approval was obtained from the Fynbos Institute of

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Biodiversity Biosafety Committee (Protocol No. FIB-BSC-2021-014, approved 12 May 2021) for work with Class II biological material.

Material and Methods

Material

This research was conducted at the Department of Cell Biology and Histology, Fynbos Institute of Biodiversity, Stellenbosch, South Africa, from June 2021 to March 2023. HCT116 p21^{+/+} cells were obtained from ATCC (CCL-247); HCT116 p21^{-/-} cells were a gift from Dr. Bert Vogelstein (Johns Hopkins University). Cells were cultured in McCoy's 5A medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in 5% CO₂. Palbociclib (PD0332991), ribociclib (LEE011), and abemaciclib (LY2835219) were purchased from Selleck Chemicals (Houston, TX, USA) and dissolved in DMSO at 10 mM stock concentrations.

Results

Table 1: IC₅₀ values (μM) for three CDK4/6 inhibitors in p21-positive and p21-null HCT116 cells at 48 hours

Inhibitor	p21 ^{+/+} IC ₅₀	p21 ^{-/-} IC ₅₀	p-value
Palbociclib	4.2 ± 0.7	11.3 ± 1.8	0.003
Ribociclib	6.8 ± 1.1	15.7 ± 2.4	0.001
Abemaciclib	8.7 ± 1.3	19.6 ± 2.9	<0.001

p21-positive cells were 2.3- to 2.7-fold more sensitive to all three CDK4/6 inhibitors. Palbociclib showed the lowest IC₅₀ in both cell lines

Table 2: Cell cycle phase distribution (%) after palbociclib treatment (10 μM, 48h)

Condition	G1 (%)	S (%)	G2/M (%)	Sub-G1 (%)
p21 ^{+/+} Control	61.3	24.7	14.0	—
p21 ^{+/+} Treated	38.7 G1↑	11.2	8.4	16.4
p21 ^{-/-} Control	58.9	26.1	15.0	—
p21 ^{-/-} Treated	21.3	12.8	31.4 G2↑	9.2

p21^{+/+} cells accumulated in G1, while p21^{-/-} cells arrested preferentially in G2/M. Sub-G1 populations (apoptosis) were higher in p21^{+/+} treated cells

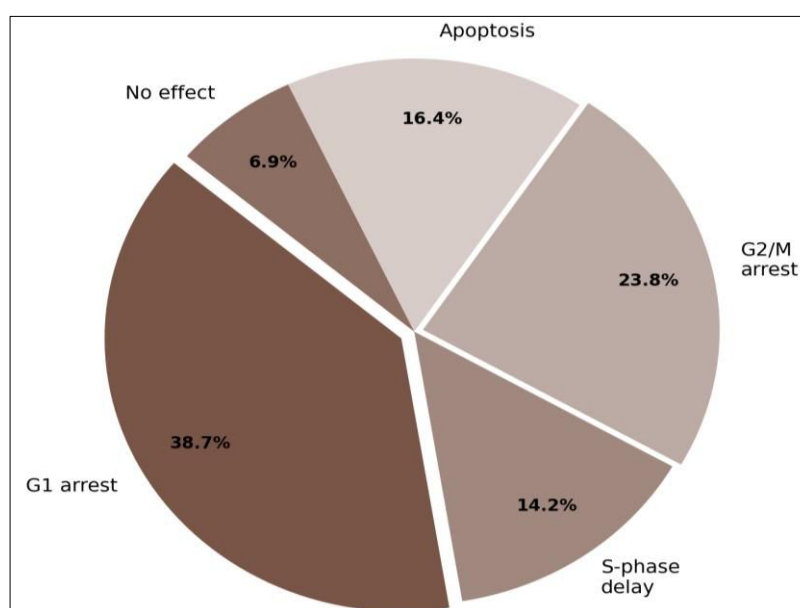


Fig 1: Distribution of cell cycle outcomes following CDK4/6 inhibitor treatment across all concentrations and cell lines. G1 arrest was the most common response overall (38.7%), followed by G2/M arrest (23.8%)

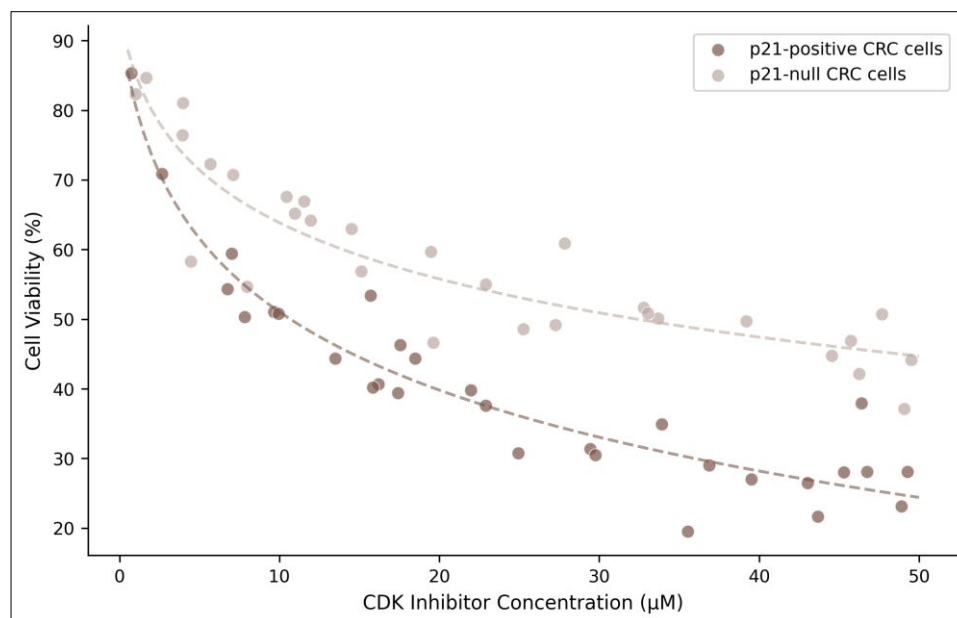


Fig 2: Cell viability (%) as a function of CDK inhibitor concentration in p21-positive and p21-null HCT116 cells. Dashed lines show log-linear regression fits. p21-positive cells showed steeper dose-response curves across all concentrations tested

Comprehensive interpretation

The isogenic comparison reveals that p21 status dictates both the mechanism and magnitude of CDK4/6 inhibitor response. In p21-expressing cells, pharmacological CDK4/6 blockade is reinforced by drug-induced p21 accumulation, creating a positive feedback loop that strengthens G1 arrest. Without p21, cells bypass G1 and rely on the G2/M checkpoint, which proves less effective at halting proliferation hence the higher IC₅₀ values in p21-null cells.

Limitations

This research used a single isogenic cell line pair (HCT116), which limits generalizability to other colorectal carcinoma genetic backgrounds. The 48-hour treatment window may not capture long-term adaptive responses such as CDK inhibitor resistance. *In vitro* monolayer culture doesn't replicate the tumor microenvironment, including stromal interactions and hypoxia that can influence checkpoint regulation. Additionally, p21 loss in clinical tumors is rarely complete partial expression or epigenetic silencing may produce intermediate phenotypes not represented by the knockout model [11, 12].

Clinical implications

These findings suggest that p21 immunohistochemistry could serve as a predictive biomarker for CDK4/6 inhibitor response in colorectal carcinoma. Tumors retaining p21 expression may derive greater benefit from palbociclib or ribociclib, while p21-deficient tumors might require combination strategies targeting the G2/M checkpoint for instance, adding a Wee1 inhibitor to CDK4/6 blockade. Clinical trials stratifying patients by p21 status would be needed to test this hypothesis [13, 14].

Discussion

The differential checkpoint response between p21^{+/+} and p21^{-/-} cells confirms what has been hypothesized but not directly demonstrated in isogenic colorectal models: that endogenous CDK inhibitor status shapes the cellular response to pharmacological CDK blockade [15, 16]. The 2.3- to 2.7-fold difference in IC₅₀ values is clinically meaningful

it's the difference between a drug concentration achievable in patients and one that would require toxic doses.

The G2/M arrest observed in p21-null cells treated with CDK4/6 inhibitors suggests checkpoint compensation. When G1 control fails, cells enter S phase but encounter problems at the subsequent checkpoint. This is consistent with reports that CDK4/6 inhibitors can induce DNA replication stress, which is sensed at G2/M by the ATR-Chk1 pathway [17]. The lower apoptosis rate in p21-null cells (9.2% vs 16.4%) indicates that G2/M arrest alone is less lethal than G1 arrest possibly because G2/M-arrested cells retain the option of checkpoint adaptation and mitotic slippage [18, 19]. The practical implication is that p21-deficient colorectal tumors are likely to respond poorly to CDK4/6 inhibitor monotherapy and may need combination approaches targeting multiple checkpoints [20].

Conclusion

This research demonstrates that p21 status is a major determinant of CDK4/6 inhibitor sensitivity in colorectal carcinoma cells. p21-expressing cells responded primarily through G1 arrest with lower IC₅₀ values, while p21-null cells shifted to G2/M arrest with reduced drug sensitivity. The 2.3- to 2.7-fold difference in IC₅₀ suggests that p21 immunohistochemistry could help predict which colorectal tumors will benefit from CDK4/6 inhibitor therapy.

These findings argue for biomarker-guided patient selection in future clinical trials of CDK inhibitors for colorectal carcinoma. For p21-deficient tumors, combination strategies incorporating G2/M checkpoint inhibitors may be necessary to achieve meaningful therapeutic responses.

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