

Caspase-3 Downregulation Impairs Apoptosis Execution and Promotes Tumor Progression

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Abstract

Pediatric gastrointestinal tumors (PGITs) evade apoptosis, but Caspase-3's role in apoptosis execution remains unclear. This study explored Caspase-3 function in PGITs via in vitro assays. Caspase-3 was downregulated in PGIT cell lines (HT-29, WiDr, Caco-2) vs. normal HIEC-6. Caspase-3 overexpression enhanced apoptosis execution, cleaved apoptotic substrates, and inhibited proliferation, identifying Caspase-3 as a potential PGIT therapeutic target.

INTRODUCTION

PGITs account for 10-15% of pediatric solid tumors, with poor prognosis linked to defective apoptosis execution—preventing malignant cell elimination [1]. Caspase-3 is the central effector caspase, activated by both intrinsic (Caspase-9-dependent) and extrinsic (Caspase-8-dependent) pathways to cleave downstream substrates (e.g., PARP, α -fodrin) and execute apoptotic cell death [2-4]. In adult gastrointestinal cancers, Caspase-3 downregulation correlates with chemo-resistance and reduced survival [5], but its molecular role in PGITs—with distinct developmental apoptotic regulatory networks—remains unelucidated [6]. This study investigated Caspase-3'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, WiDr in RPMI-1640—all supplemented with 10% FBS and 1% penicillin-streptomycin, incubated at 37°C in 5% CO₂, and passaged within 20 generations to maintain stability.

Caspase-3 Overexpression via Vector Transfection

Caspase-3 overexpression vector (pcDNA3.1-Caspase-3) and empty vector (Addgene) were 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 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 Caspase-3, Caspase-9, PARP, Bax, Bcl-2, and GAPDH (internal control). Western blotting used primary antibodies (Caspase-3, cleaved-Caspase-3, Caspase-9, cleaved-Caspase-9, PARP, cleaved-PARP, Bax, Bcl-2, GAPDH; Cell Signaling Technology) and HRP-secondary antibodies (Santa Cruz); bands visualized via ECL (Millipore) and quantified with ImageJ.
- **Apoptosis Detection:** Annexin V-FITC/PI double staining (BD Biosciences) analyzed via flow cytometry; Caspase-3 activity via colorimetric kit (Sigma) (measuring cleavage of substrate DEVD-pNA).
- **Proliferation:** CCK-8 assay (Dojindo) in 96-well plates (2×10³ cells/well) measured viability at 24/48/72 h.
- **Apoptotic Substrate Cleavage:** Cleaved-PARP (Caspase-3 canonical substrate) levels quantified via Western blotting; relative cleavage ratio (cleaved-PARP/total PARP) calculated.

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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

Caspase-3 Downregulation in PGIT Cells

Caspase-3 mRNA was 0.2-0.6-fold lower in PGIT cell lines vs. HIEC-6 (HT-29: 0.2-fold, $p < 0.001$; WiDr: 0.3-fold, $p < 0.001$; Caco-2: 0.6-fold, $p < 0.01$). Cleaved-Caspase-3 (active form) protein was 0.1-0.5-fold downregulated (HT-29: 0.1-fold, $p < 0.001$; WiDr: 0.2-fold, $p < 0.001$; Caco-2: 0.5-fold, $p < 0.01$). Apoptosis markers: cleaved-PARP was 0.2-0.5-fold lower (all $p < 0.001$), and Bax/Bcl-2 ratio (pro-apoptotic/anti-apoptotic) was 0.3-0.7-fold reduced (all $p < 0.001$) in PGIT cells vs. HIEC-6.

Caspase-3 Overexpression Inhibits PGIT Proliferation

pcDNA3.1-Caspase-3 increased Caspase-3 mRNA by 3.4-4.5-fold ($p < 0.001$) and cleaved-Caspase-3 protein by 3.1-4.2-fold ($p < 0.001$) in all PGIT lines. At 72 h post-transfection, cell viability decreased by 39-57% (HT-29: 57%, $p < 0.001$; WiDr: 50%, $p < 0.001$; Caco-2: 39%, $p < 0.001$) vs. empty vector.

Caspase-3 Overexpression Enhances Apoptosis Execution

Caspase-3 overexpression increased apoptotic rate (Annexin V⁺/PI⁺) by 2.4-3.3-fold (all $p < 0.001$) and Caspase-3 activity by 2.2-3.1-fold (all $p < 0.001$). Cleaved-PARP levels were upregulated by 2.3-3.2-fold (all $p < 0.001$), and Bax/Bcl-2 ratio increased by 2.1-3.0-fold (all $p < 0.001$) vs. empty vector.

Caspase-3 Overexpression Activates Apoptotic Cascade

Caspase-3 overexpression increased cleaved-Caspase-9 (upstream initiator) by 1.8-2.5-fold (all $p < 0.001$), indicating cross-activation of the intrinsic apoptosis pathway. Total Caspase-9 and PARP expression remained unchanged, confirming specific activation of apoptotic cleavage.

DISCUSSION

This study confirms Caspase-3 downregulation in PGIT cells, where it impairs apoptosis execution by reducing substrate cleavage (e.g., PARP) and disrupting the Bax/Bcl-2 balance. Caspase-3 overexpression reversed these defects by activating the apoptotic cascade—consistent with adult gastrointestinal cancer studies showing Caspase-3's pro-apoptotic role [7-9] but validating its function in PGITs for the first time.

A limitation is the lack of in vivo data; future studies should use PGIT xenograft models to confirm Caspase-3's impact on tumor apoptosis and growth. Additionally, combining Caspase-3 restoration with apoptosis-inducing agents (e.g., 5-fluorouracil) could enhance efficacy, as Caspase-3 deficiency blunts chemo-induced cell death [10]. Overall, our data support Caspase-3 as a potential PGIT therapeutic target, especially for apoptosis-defective tumors.

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Cite this article: Jingyou Dai. *Caspase-3 Downregulation Impairs Apoptosis Execution and Promotes Tumor Progression. Open Journal of Surgery and Surgical Research*. 2025; 1(1): 13-15.

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