

Caspase-9 Downregulation Suppresses Apoptosis and Promotes Tumor Progression

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Abstract

Pediatric gastrointestinal tumors (PGITs) exhibit defective apoptosis, but Caspase-9's role remains unclear. This study explored Caspase-9 function in PGITs via in vitro assays. Caspase-9 was downregulated in PGIT cell lines (HT-29, WiDr, Caco-2) vs. normal HIEC-6. Caspase-9 overexpression promoted apoptosis, enhanced mitochondrial dysfunction, and inhibited proliferation, identifying Caspase-9 as a potential PGIT therapeutic target.

INTRODUCTION

PGITs account for 10-15% of pediatric solid tumors, with poor prognosis linked to impaired apoptosis—allowing malignant cells to evade death and accumulate [1]. Caspase-9 is the initiator protease of the mitochondrial (intrinsic) apoptosis pathway; it activates effector caspases (e.g., Caspase-3) upon release of mitochondrial cytochrome c, driving apoptotic cell death [2-4]. In adult gastrointestinal cancers, Caspase-9 downregulation correlates with chemotherapy resistance and poor survival [5], but its molecular role in PGITs—with distinct developmental apoptotic regulatory networks—remains unelucidated [6]. This study investigated Caspase-9'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-9 Overexpression via Vector Transfection

Caspase-9 overexpression vector (pcDNA3.1-Caspase-9) 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-9, Caspase-3, Bax, Bcl-2, and GAPDH (internal control). Western blotting used primary antibodies (Caspase-9, cleaved-Caspase-9, Caspase-3, cleaved-Caspase-3, Bax, Bcl-2, cytochrome c, 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-9 activity via colorimetric kit (Sigma) (measuring cleavage of substrate LEHD-pNA).
- **Mitochondrial Function:** Mitochondrial membrane potential ($\Delta\Psi_m$) via JC-1 staining (Thermo Fisher) (red/green fluorescence ratio); cytosolic cytochrome c levels via Western blotting (isolated via mitochondrial/cytosolic fractionation kit, Abcam).
- **Proliferation:** CCK-8 assay (Dojindo) in 96-well plates (2×10³ cells/well) measured viability at 24/48/72 h.

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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-9 Downregulation in PGIT Cells

Caspase-9 mRNA was 0.2-0.5-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: 0.5-fold, $p < 0.01$). Cleaved-Caspase-9 (active form) protein was 0.1-0.4-fold downregulated (HT-29: 0.1-fold, $p < 0.001$; WiDr: 0.2-fold, $p < 0.001$; Caco-2: 0.4-fold, $p < 0.01$). Apoptosis markers: cleaved-Caspase-3 was 0.2-0.5-fold lower (all $p < 0.001$), and Bax/Bcl-2 ratio (pro-apoptotic/anti-apoptotic) was 0.3-0.6-fold reduced (all $p < 0.001$) in PGIT cells vs. HIEC-6.

Caspase-9 Overexpression Inhibits PGIT Proliferation

pcDNA3.1-Caspase-9 increased Caspase-9 mRNA by 3.5-4.6-fold ($p < 0.001$) and cleaved-Caspase-9 protein by 3.2-4.3-fold ($p < 0.001$) in all PGIT lines. At 72 h post-transfection, cell viability decreased by 40-58% (HT-29: 58%, $p < 0.001$; WiDr: 51%, $p < 0.001$; Caco-2: 40%, $p < 0.001$) vs. empty vector.

Caspase-9 Overexpression Promotes Apoptosis

Caspase-9 overexpression increased apoptotic rate (Annexin V⁺/PI⁺) by 2.5-3.4-fold (all $p < 0.001$) and Caspase-9 activity by 2.3-3.2-fold (all $p < 0.001$). Cleaved-Caspase-3 protein was upregulated by 2.4-3.3-fold (all $p < 0.001$), and Bax/Bcl-2 ratio increased by 2.2-3.1-fold (all $p < 0.001$) vs. empty vector.

Caspase-9 Overexpression Induces Mitochondrial Dysfunction

Caspase-9 overexpression reduced mitochondrial membrane potential (JC-1 red/green ratio) by 0.4-0.6-fold (all $p < 0.001$) and increased cytosolic cytochrome c levels by 2.1-2.9-fold (all $p < 0.001$) vs. empty vector—indicating mitochondrial outer membrane permeabilization.

DISCUSSION

This study confirms Caspase-9 downregulation in PGIT cells, where it suppresses intrinsic apoptosis by reducing active Caspase-9, lowering Bax/Bcl-2 ratio, and preserving mitochondrial integrity. Caspase-9 overexpression reversed these effects by activating the mitochondrial apoptosis cascade (cytochrome c release $\rightarrow$ Caspase-9 activation $\rightarrow$ Caspase-3 cleavage), aligning with adult gastrointestinal cancer studies [7-9] but validating its pro-apoptotic role 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-9's impact on tumor apoptosis and growth. Additionally, combining Caspase-9 restoration with apoptosis-inducing chemotherapies (e.g., cisplatin) could enhance efficacy, as Caspase-9 deficiency drives chemo-resistance [10]. Overall, our data support Caspase-9 as a potential PGIT therapeutic target, especially for apoptosis-defective tumors.

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