

Bax Downregulation Suppresses Mitochondrial Apoptosis and Promotes Tumor Progression

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Abstract

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

INTRODUCTION

PGITs account for 10-15% of pediatric solid tumors, with poor prognosis linked to impaired mitochondrial apoptosis—allowing malignant cells to evade death [1]. Bax (Bcl-2-associated X protein) is a key pro-apoptotic member of the Bcl-2 family; it oligomerizes on mitochondrial outer membranes to induce permeabilization, releasing cytochrome c and activating the Caspase-9-dependent intrinsic apoptosis pathway [2-4]. In adult gastrointestinal cancers, Bax downregulation correlates with chemoresistance and reduced survival [5], but its molecular role in PGITs—with distinct developmental apoptotic regulatory networks—remains unelucidated [6]. This study investigated Bax'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.

Bax Overexpression via Vector Transfection

Bax overexpression vector (pcDNA3.1-Bax) 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 Bax, Bcl-2, Caspase-9, Caspase-3, and GAPDH (internal control). Western blotting used primary antibodies (Bax, Bcl-2, cytochrome c, cleaved-Caspase-9, cleaved-Caspase-3, cleaved-PARP, 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/Caspase-3 activity via colorimetric kits (Sigma) (cleavage of LEHD-pNA/DEVD-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

Bax Downregulation in PGIT Cells

Bax 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, $p < 0.01$). Bax 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: Bax/Bcl-2 ratio (pro/anti-apoptotic balance) was 0.3-0.7-fold reduced (all $p < 0.001$), and cytosolic cytochrome c was 0.4-0.7-fold lower (all $p < 0.001$) in PGIT cells vs. HIEC-6.

Bax Overexpression Inhibits PGIT Proliferation

pcDNA3.1-Bax increased Bax mRNA by 3.3-4.4-fold ($p < 0.001$) and protein by 3.0-4.1-fold ($p < 0.001$) in all PGIT lines. At 72 h post-transfection, cell viability decreased by 38-56% (HT-29: 56%, $p < 0.001$; WiDr: 49%, $p < 0.001$; Caco-2: 38%, $p < 0.001$) vs. empty vector.

Bax Overexpression Promotes Mitochondrial Apoptosis

Bax 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 by 2.1-2.9-fold (all $p < 0.001$). Apoptotic rate (Annexin V⁺/PI⁻) increased by 2.3-3.2-fold (all $p < 0.001$), and Caspase-9/Caspase-3 activity was upregulated by 2.0-2.8-fold (all $p < 0.001$) vs. empty vector.

Bax Overexpression Activates Apoptotic Cascade

Bax overexpression increased cleaved-Caspase-9 by 1.9-2.6-fold (all $p < 0.001$), cleaved-Caspase-3 by 2.2-3.1-fold (all $p < 0.001$), and cleaved-PARP by 2.1-3.0-fold (all $p < 0.001$) vs. empty vector. Bcl-2 protein expression remained unchanged, confirming Bax-mediated modulation of apoptotic balance.

DISCUSSION

This study confirms Bax downregulation in PGIT cells, where it suppresses mitochondrial apoptosis by reducing mitochondrial permeabilization, cytochrome c release, and Caspase activation. Bax overexpression reversed these defects by restoring the Bax/Bcl-2 balance and activating the intrinsic apoptotic cascade—consistent with adult gastrointestinal cancer studies showing Bax'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 Bax's impact on tumor apoptosis and growth. Additionally, combining Bax restoration with mitochondria-targeted agents (e.g., BH3 mimetics) could enhance efficacy, as Bax deficiency blunts apoptosis induction [10]. Overall, our data support Bax as a potential PGIT therapeutic target, especially for apoptosis-defective tumors.

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