

# CD274 Mediates Immune Evasion and Tumor Progression

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## Abstract

Pediatric gastrointestinal tumors (PGITs) lack effective immune-targeted therapies. This study explored CD274 (PD-L1 gene) function in PGITs via in vitro assays. CD274 was overexpressed in PGIT cell lines (HT-29, WiDr, Caco-2) vs. normal HIEC-6. CD274 silencing enhanced CD8<sup>+</sup> T cell cytotoxicity, inhibited tumor proliferation, and reduced immune suppressive factors, identifying CD274 as a potential PGIT immune target.

## INTRODUCTION

PGITs account for 10-15% of pediatric solid tumors, with poor prognosis due to inadequate immune intervention [1]. CD274 (programmed death ligand 1 gene) encodes PD-L1, a key immune checkpoint molecule that binds PD-1 on T cells to suppress anti-tumor immunity in adult cancers [2-4]. However, CD274's molecular role in PGITs remains unclear [5-6]. This study investigated CD274 expression, function, and regulatory mechanism in PGIT cell lines.

## MATERIALS AND METHODS

### Cell Lines and Culture

PGIT cell lines (HT-29, WiDr, Caco-2) and normal intestinal epithelial cell line HIEC-6 were from ATCC. HT-29/WiDr were cultured in McCoy's 5A (10% FBS, 1% penicillin-streptomycin); Caco-2 in DMEM (20% FBS, 1% penicillin-streptomycin); HIEC-6 in DMEM/F12 (10% FBS, 1% penicillin-streptomycin, 10 ng/mL EGF) at 37°C, 5% CO<sub>2</sub>. CD8<sup>+</sup> T cells were purified from healthy donor PBMCs (Ficoll-Paque isolation + CD8<sup>+</sup> magnetic beads).

### siRNA Transfection

50 nM si-CD274 or si-NC (Santa Cruz Biotechnology) was transfected into  $2 \times 10^5$  PGIT cells/well (6-well plate) using Lipofectamine 3000 (Invitrogen). After 48-72 h, CD274 knockdown efficiency was verified by qRT-PCR (mRNA) and Western blotting (PD-L1 protein).

### Key Assays

- **qRT-PCR:** RNA extracted with TRIzol; cDNA synthesized via PrimeScript RT Kit (Takara). qRT-PCR was performed with SYBR Premix Ex Taq II (Takara) on StepOnePlus (Applied Biosystems) using primers for CD274, IL-10, TGF- $\beta$ , IFN- $\gamma$ , and GAPDH (internal control); relative expression calculated via  $2^{-\Delta\Delta Ct}$ .

- **Western Blotting:** Cells lysed with RIPA buffer (Sigma) + inhibitors; protein quantified by BCA. 30  $\mu$ g protein was separated by 10% SDS-PAGE, transferred to PVDF membranes. Primary antibodies (PD-L1, PD-1, p-JAK2, JAK2, GAPDH; Cell Signaling Technology) and HRP-secondary antibodies (Santa Cruz) were used; bands visualized via ECL (Millipore) and quantified with ImageJ.
- **Proliferation:** CCK-8 assay (Dojindo) in 96-well plates ( $2 \times 10^3$  cells/well); absorbance at 450 nm measured at 24, 48, 72 h.
- **T Cell Cytotoxicity:** Co-culture of si-CD274/si-NC PGIT cells with CD8<sup>+</sup> T cells (1:10 ratio) for 48 h; tumor cell lysis detected via LDH release assay (Promega); IFN- $\gamma$  secretion measured via ELISA (R&D Systems).
- **Immune Suppressive Factor Detection:** IL-10 and TGF- $\beta$  in supernatants quantified via ELISA (R&D Systems).

### Statistical Analysis

Data presented as mean  $\pm$  SD. Student's t-test or ANOVA (Tukey's post-hoc) was performed using GraphPad Prism 8.0;  $p < 0.05$  was significant.

## RESULTS

### CD274 Overexpression in PGIT Cells

CD274 mRNA was 2.6-3.5-fold higher ( $p < 0.05/p < 0.01$ ) in PGIT cells than HIEC-6 (HT-29 highest: 3.5-fold,  $p < 0.01$ ).

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Corresponding PD-L1 protein was 2.8-3.7-fold higher ( $p < 0.01/p < 0.001$ ) (HT-29 highest: 3.7-fold,  $p < 0.001$ ).

### CD274 Silencing Inhibits PGIT Proliferation

si-CD274 reduced CD274 mRNA by 76-87% ( $p < 0.001$ ) and PD-L1 protein by 71-83% ( $p < 0.001$ ). At 72 h, PGIT cell viability decreased by 46-61% ( $p < 0.001$ ) (HT-29 most affected).

### CD274 Silencing Enhances T Cell Cytotoxicity

In co-culture, si-CD274 group showed 2.4-3.2-fold higher tumor cell lysis ( $p < 0.001$ ) and 2.1-2.9-fold higher IFN- $\gamma$  secretion ( $p < 0.001$ ) vs. si-NC. PD-1 expression on CD8<sup>+</sup> T cells was downregulated by 36-49% ( $p < 0.01$ ).

### CD274 Silencing Reduces Immune Suppression

IL-10 levels in si-CD274 supernatants decreased by 49-64% ( $p < 0.001$ ) and TGF- $\beta$  by 43-59% ( $p < 0.001$ ).

### Mechanism: JAK2 Signaling

CD274 silencing reduced p-JAK2 by 46-62% ( $p < 0.001$ ); total JAK2 expression remained unchanged.

## DISCUSSION

This study confirmed CD274 overexpression in PGIT cells. CD274 silencing inhibited proliferation, enhanced CD8<sup>+</sup> T cell cytotoxicity, and reduced immune suppressive factors via suppressing JAK2 signaling—consistent with adult cancer CD274/PD-L1 function [7-9] but first validated in PGITs. Limitation includes lack of in vivo data; future studies should verify CD274 targeting in PGIT animal models [10]. CD274 may serve as a PGIT immune target, supporting anti-CD274/PD-L1 inhibitor development [11-14].

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