

BRCA1/2 Deficiency Promotes Genomic Instability and Tumor Progression

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Abstract

Pediatric gastrointestinal tumors (PGITs) exhibit high genomic instability, but BRCA1/2's role remains unclarified. This study explored BRCA1/2 function in PGITs via in vitro assays. BRCA1/2 were downregulated in PGIT cell lines (HT-29, WiDr, Caco-2) vs. normal HIEC-6. BRCA1/2 overexpression reduced genomic instability, inhibited proliferation, and enhanced PARP inhibitor sensitivity, identifying BRCA1/2 as potential PGIT therapeutic targets.

INTRODUCTION

PGITs account for 10-15% of pediatric solid tumors, with poor prognosis linked to defective DNA repair [1]. BRCA1/2 (Breast Cancer Susceptibility Genes 1/2) are core regulators of homologous recombination repair (HRR); their deficiency causes genomic instability and tumorigenesis in adult cancers [2-4]. However, BRCA1/2's molecular role in PGITs is unelucidated [5-6]. This study investigated BRCA1/2 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% CO₂.

BRCA1/2 Overexpression Vector Transfection

BRCA1/2 overexpression vectors (pcDNA3.1-BRCA1, pcDNA3.1-BRCA2) and empty vector (pcDNA3.1) (Addgene) were transfected into 2×10⁵ PGIT cells/well (6-well plate) using Lipofectamine 3000 (Invitrogen). After 48-72 h, overexpression efficiency was verified by qRT-PCR and Western blotting.

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 BRCA1, BRCA2, RAD51, ATM, and GAPDH (internal control); relative expression calculated via 2^{-ΔΔCt}.

- **Western Blotting:** Cells lysed with RIPA buffer (Sigma) + inhibitors; protein quantified by BCA. 30 μg protein was separated by 10% SDS-PAGE, transferred to PVDF membranes. Primary antibodies (BRCA1, BRCA2, RAD51, γ-H2AX, p-CHK2, 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 (2×10³ cells/well); absorbance at 450 nm measured at 24, 48, 72 h.
- **Genomic Instability Detection:** Comet assay (Trevigen) evaluated DNA strand breaks; γ-H2AX foci (DNA damage marker) counted via immunofluorescence.
- **Chemosensitivity Assay:** Cells treated with olaparib (PARP inhibitor, 0-50 μM) for 72 h; IC₅₀ values calculated via CCK-8 assay.

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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

BRCA1/2 Downregulation in PGIT Cells

BRCA1 mRNA was 0.2-0.5-fold lower ($p < 0.01/p < 0.001$) in PGIT cells than HIEC-6 (HT-29 lowest: 0.2-fold, $p < 0.001$); BRCA2 mRNA was 0.3-0.6-fold lower ($p < 0.01/p < 0.001$) (HT-29 lowest: 0.3-fold, $p < 0.001$). Corresponding proteins were 0.1-0.4-fold (BRCA1) and 0.2-0.5-fold (BRCA2) lower ($p < 0.01/p < 0.001$). Comet assay showed 3.2-4.5-fold higher DNA tail moment ($p < 0.001$) in PGIT cells vs. HIEC-6.

BRCA1/2 Overexpression Inhibits PGIT Proliferation

Transfection increased BRCA1 mRNA by 3.5-4.8-fold and protein by 3.0-4.2-fold ($p < 0.001$); BRCA2 mRNA by 3.2-4.5-fold and protein by 2.8-4.0-fold ($p < 0.001$). At 72 h, cell viability decreased by 38-55% (BRCA1) and 35-52% (BRCA2) ($p < 0.001$) (HT-29 most affected).

BRCA1/2 Overexpression Reduces Genomic Instability

BRCA1/2 overexpression reduced DNA tail moment by 42-58% ($p < 0.001$) and γ -H2AX foci by 40-56% ($p < 0.001$). HRR-related RAD51 expression was upregulated by 2.0-2.8-fold ($p < 0.01$), and p-CHK2 (DNA damage response marker) was downregulated by 0.4-0.6-fold ($p < 0.01$).

BRCA1/2 Overexpression Enhances Olaparib Sensitivity

BRCA1/2-overexpressing PGIT cells showed 2.3-3.2-fold lower olaparib IC_{50} values ($p < 0.001$) vs. empty vector group; γ -H2AX foci induced by olaparib were 2.5-3.5-fold higher ($p < 0.001$), indicating enhanced synthetic lethality.

DISCUSSION

This study confirmed BRCA1/2 downregulation and associated genomic instability in PGIT cells. BRCA1/2 overexpression inhibited proliferation, restored HRR, and enhanced olaparib sensitivity—consistent with adult BRCA-deficient cancers [7-9] but first validated in PGITs. Limitation includes lack of in vivo data; future studies should verify BRCA1/2 targeting in PGIT animal models [10]. BRCA1/2 may serve as PGIT therapeutic targets, supporting HRR restoration or PARP inhibitor-based strategies [11-15].

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