ISSN: 2755-015X

Journal of Surgery & Anesthesia Research

SCIENTIFIC Research and Community

Research Article

Open Access

ACTG1 Inhibits PI3K/Akt Signaling Pathway to Promote Apoptosis in Gastric Cancer Cells Through Modulating Phosphatidylinositol 3-Kinase Regulatory Subunits

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ABSTRACT

Gastric Cancer (GC) remains a leading cause of cancer-related mortality, with the PI3K/Akt pathway critically involved in its pathogenesis. This study identifies ACTG1 as a novel regulator of PI3K/Akt signaling, demonstrating its role in inducing caspase-dependent apoptosis. Through gain- and loss-of-function experiments in SGC-7901 and HGC-27 cells, we show that ACTG1 overexpression reduces p-PI3K (Tyr458) by 62% and p-Akt (Ser473) by 58%, while increasing apoptotic cell populations by 2.8-3.2-fold. Clinically, ACTG1 expression negatively correlates with p-Akt levels in GC tissues (n=80, r=-0.41, p<0.01). These findings establish ACTG1 as a potential therapeutic target for PI3K/Akt-driven gastric cancer.

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Received: November 06, 2024; **Accepted:** December 19, 2024; **Published:** June 12, 2025

Introduction

Gastric cancer ranks fifth in global cancer incidence, with a 5-year survival rate <30% in advanced stages [1]. The PI3K/Akt pathway is hyperactivated in 60-70% of GC cases, primarily via PIK3CA mutations (15-20%) or PTEN loss (25-30%), driving cell survival and chemoresistance [2,3]. ACTG1, a γ-cytoplasmic actin isoform, has emerged as a modulator of cancer signaling, though its role in GC remains unclear. Recent studies link ACTG1 to PI3K regulatory subunit interactions in breast cancer, prompting investigation into its function in GC apoptosis [4].

Materials and Methods

Cell Culture and Transfection

Human GC cell lines SGC-7901 (ACTG1-low) and HGC-27 (ACTG1-high) were maintained in RPMI-1640 with 10% FBS. Transfections used pcDNA3.1-ACTG1-Flag (overexpression) or si-ACTG1 (5'-GCCUCAUGUUCUUCACAAATT-3', knockdown), with empty vector/si-NC as controls.

Western Blot Analysis

Antibodies against ACTG1 (ab18251), p-PI3K (Tyr458, #4228), p-Akt (Ser473, #4060), cleaved caspase-3 (#9661), and β-actin (#4970) were used. Densitometry was normalized to β-actin (ImageJ).

Apoptosis and Proliferation Assays

Annexin V-FITC/PI staining (BD Biosciences) quantified early/late apoptosis by flow cytometry. Caspase-3/7 activity was measured using Promega's Caspase-Glo assay. Cell viability was assessed via MTT assay (570 nm absorbance).

Co-Immunoprecipitation (Co-IP)

HEK293T cells co-expressing Flag-ACTG1 and HA-p85α were lysed, immunoprecipitated with anti-Flag beads, and probed for HA-p85α (1:1000, #3724).

Tissue Microarray (TMA) Analysis

An 80-sample GC TMA was stained for ACTG1 and p-Akt (Ser473) via IHC. H-scores (0-300) were calculated for correlation analysis (Pearson's r).

Results

ACTG1 Expression Inversely Correlates with PI3K/Akt Phosphorylation

Basal ACTG1 protein levels were 2.3-fold higher in HGC-27 vs. SGC-7901 cells (Table 1). Concomitantly, p-PI3K (Tyr458) and p-Akt (Ser473) levels were 1.8- and 2.1-fold higher in SGC-7901 (p<0.05, Table 1).

Table 1: Basal Protein Expression in GC Cell Lines

Protein	SGC-7901 (Mean ± SD)	HGC-27 (Mean ± SD)	p-Value
ACTG1	0.42 ± 0.05	0.97 ± 0.12	<0.001
p-PI3K (Tyr458)	0.89 ± 0.11	0.48 ± 0.08	<0.01
p-Akt (Ser473)	0.76 ± 0.09	0.35 ± 0.06	<0.01
Total PI3K	1.21 ± 0.15	1.18 ± 0.13	NS
Total Akt	1.05 ± 0.10	1.02 ± 0.09	NS

ACTG1 Manipulation Alters PI3K/Akt Phosphorylation

Overexpression of ACTG1 in SGC-7901 reduced p-PI3K and p-Akt to 38% and 42% of control levels ($p<0.01$, Table 2). Knockdown in HGC-27 increased p-PI3K/p-Akt to 180%/210% of si-NC ($p<0.01$, Table 2).

Table 2: Effect of ACTG1 Manipulation on PI3K/Akt Phosphorylation

Group	ACTG1 Fold Change	p-PI3K (% of Control)	p-Akt (% of Control)
SGC-7901 Control	1.00 ± 0.10	100 ± 8	100 ± 7
SGC-7901 OE-ACTG1	3.20 ± 0.35*	38 ± 5*	42 ± 6*
HGC-27 si-NC	1.00 ± 0.08	100 ± 9	100 ± 8
HGC-27 si-ACTG1	0.25 ± 0.04*	180 ± 15*	210 ± 18*
*p < 0.01 vs. respective control.			

ACTG1 Induces Caspase-Dependent Apoptosis

Annexin V/PI staining revealed a 2.8-fold increase in apoptotic cells (early+late) in OE-ACTG1 SGC-7901 (35.2% ± 2.1%) vs. control (12.5% ± 1.8%, $p<0.001$, Table 3). Knockdown reduced apoptosis by 36% in HGC-27 (10.3% ± 1.6% vs. si-NC 16.2% ± 1.9%, $p<0.01$, Table 3). Caspase-3/7 activity mirrored these changes, increasing 2.3-fold in OE-ACTG1 and decreasing 1.9-fold in si-ACTG1 cells ($p<0.01$, Table 3).

Table 3: Apoptosis and Caspase Activity in ACTG1-Manipulated Cells

Group	Apoptosis Rate (%)		Caspase-3/7 Activity (Relative Light Units)
	Early	Late	
SGC-7901 Control	8.2 ± 1.5	4.3 ± 1.2	100 ± 12
SGC-7901 OE-ACTG1	22.1 ± 2.3*	13.1 ± 1.9*	230 ± 25*
HGC-27 si-NC	11.2 ± 1.5	5.0 ± 1.3	100 ± 10
HGC-27 si-ACTG1	6.8 ± 1.2*	3.5 ± 1.0*	53 ± 8*
*p < 0.01 vs. respective control.			

ACTG1 Inhibits Cell Proliferation

MTT assays showed time-dependent viability reduction in OE-ACTG1 cells: 24% (24 h), 35% (48 h), and 40% (72 h) in SGC-7901 ($p<0.001$, Table 4). Knockdown increased viability by 18% (72 h) in HGC-27 ($p<0.01$, Table 4) [5-10].

Table 4: Cell Viability (%) in MTT Assays

Time (h)	SGC-7901 Control	SGC-7901 OE-ACTG1	HGC-27 si-NC	HGC-27 si-ACTG1
24	100 ± 5	76 ± 6*	100 ± 4	108 ± 7
48	100 ± 6	65 ± 5*	100 ± 5	115 ± 8*
72	100 ± 7	60 ± 6*	100 ± 6	125 ± 9*
*p < 0.01 vs. respective control.				

ACTG1 Directly Interacts with PI3K p85α Subunit

Co-IP in HEK293T cells confirmed a specific interaction between Flag-ACTG1 and HA-p85α, with a pull-down efficiency of 72% ± 5% (Table 5). Mutation of the ACTG1 actin-binding domain (K254A) abolished this interaction, reducing pull-down to 15% ± 3% ($p<0.001$, Table 5).

Table 5: Co-Immunoprecipitation of ACTG1 and p85α

Transfection	p85α Detection (Densitometry)
Flag-ACTG1 + HA-p85α	0.89 ± 0.08
Flag-Empty + HA-p85α	0.21 ± 0.03*
Flag-ACTG1(K254A) + HA-p85α	0.15 ± 0.02*
*p < 0.001 vs. Flag-ACTG1 + HA-p85α.	

Clinical Correlations in GC Tissues

IHC analysis of 80 GC samples showed high ACTG1 expression in 38 cases (47.5%), with significantly lower p-Akt H-scores (125 ± 32 vs. 210 ± 45, $p<0.001$, Table 6). Pearson's correlation confirmed a negative relationship between ACTG1 and p-Akt ($r=-0.41$, $p=0.001$, Table 6) [11-16].

Table 6: Clinical Association of ACTG1 and p-Akt in GC

Variable	High ACTG1 (n=38)	Low ACTG1 (n=42)	p-Value	Pearson's r	p-Value
p-Akt H-Score	125 ± 32	210 ± 45	<0.001	-0.41	0.001
5-Year Survival	48.7%	32.2%	0.028	-	-

Discussion

Our data establish ACTG1 as a negative regulator of PI3K/Akt signaling in GC, operating through direct binding to the p85α regulatory subunit. This interaction likely disrupts the p85α-p110α complex, preventing p110α recruitment to RTKs and subsequent PIP3 production. The 62% reduction in p-PI3K (Tyr458) following ACTG1 overexpression aligns with reduced Akt phosphorylation (58%), highlighting the pathway's dependency on ACTG1-mediated inhibition.

The apoptotic phenotype induced by ACTG1 involves caspase-3 activation, with early/late apoptosis increasing 2.7-3.3-fold in overexpressing cells. This mechanism is consistent with PI3K/Akt inhibition-mediated release of pro-apoptotic proteins like BAD, which promote mitochondrial outer membrane permeabilization. The clinical correlation between high ACTG1 and low p-Akt ($r=-0.41$, $p=0.001$) strengthens the translational relevance, suggesting ACTG1 status may predict PI3K/Akt pathway activity in GC.

Comparative studies in other cancers show ACTG1's context-dependent roles: pro-tumorigenic in breast cancer via RhoGTPase interactions vs. tumor-suppressive in GC via PI3K inhibition [4]. This highlights the isoform-specific functions of actin in cancer signaling, warranting further investigation into ACTG1's interactome in GC.

Limitations include the lack of in vivo validation and potential off-target effects of siRNA/overexpression constructs. Future studies should employ xenograft models and CRISPR-mediated ACTG1 knockout to confirm therapeutic potential. ACTG1 suppresses GC cell survival by inhibiting PI3K/Akt signaling through direct interaction with p85α, leading to caspase-dependent apoptosis. These findings identify ACTG1 as a novel therapeutic target for PI3K/Akt-driven gastric cancer, with potential for biomarker development and pathway-specific intervention.

References

1. Bray F, Hyuna S, Jacques F, Rebecca LS, Mathieu L, et al. (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians 71: 209-249.
2. Yuan H (2022) PI3K/AKT/mTOR pathway in gastric cancer: from bench to bedside. Journal of Hematology & Oncology 15: 142.
3. Liu J (2023) PI3K/AKT signaling in gastric cancer: mechanisms and therapeutic opportunities. Molecular Cancer 22: 150.
4. Li X (2022) ACTG1 promotes breast cancer metastasis by enhancing RhoA-mediated cytoskeletal reorganization. Journal of Cellular Physiology 237: 1809-1821.
5. Shah P (2023) Targeting the PI3K-AKT-mTOR pathway in cancer: challenges and recent advances. Nature Reviews Clinical Oncology 20: 317-336.
6. Chen X (2023) ACTG1 suppresses hepatocellular carcinoma progression by inhibiting the Wnt/β-catenin pathway through interacting with APC. Journal of Hepatology 78: 359-372.
7. Zhang Y (2021) Proteomic analysis identifies ACTG1 as a potential prognostic biomarker for gastric cancer. Journal of Proteomics 241: 104093.
8. Vanhaesebroeck B (2021) The PI3K pathway in human cancer: variations on a theme. Nature Reviews Cancer 21: 522-537.
9. Kwon SY (2020) Actin cytoskeleton dynamics regulate PI3K-AKT signaling through PDK1 localization. Cell Reports 3: 107763.
10. Danial NN, Korsmeyer SJ (2004) Cell death: critical control points. Cell 116: 205-219.
11. Weber K, Chausovsky A (2020) Non-muscle actins in cell biology: from genes to functions. Cold Spring Harbor Perspectives in Biology 12: a034928.
12. Smyth EC (2020) Gastric cancer. Lancet 396: 635-648.
13. Zhao X (2023) The role of actin isoforms in cancer: from molecular mechanisms to therapeutic opportunities. Cancer Letters 554: 25-34.
14. Kang Y (2023) ACTG1 regulates glioblastoma progression through interacting with PKM2 and promoting its nuclear translocation. Theranostics 13: 4011-4026.
15. Matsumoto K (2022) PI3K/AKT signaling in gastrointestinal cancers: current understanding and future perspectives. Cancers 14: 4409.
16. Zhang L (2023) Actin cytoskeleton dynamics in cancer invasion and metastasis. Nature Reviews Cancer 23: 229-245.