American Journal of Men's Health
September-October 1–12
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DOI: 10.1177/15579883241273305
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KLF1 Activates RAC3 to Mediate Fatty Acid Synthesis and Enhance Cisplatin Resistance in Bladder Cancer Cells

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Abstract

While cisplatin remains a frontline treatment for bladder cancer (BCa), the onset of resistance greatly hampers its effectiveness. RAC3 is closely linked to chemoresistance in cancer cells, but its specific role in cisplatin resistance within BCa is still elusive. RAC3 expression in BCa was analyzed using bioinformatics and quantitative polymerase chain reaction (qPCR). The gene set enrichment analysis (GSEA) identified RAC3-enriched pathways and the correlation between RAC3 and fatty acid synthase (FASN), a gene involved in fatty acid synthesis. Potential upstream transcription factors of RAC3 were predicted and their interaction with RAC3 was confirmed via dual-luciferase and chromatin immunoprecipitation (ChIP) assays. T24/DDP, a cisplatin-resistant BCa cell line, was established to probe into the regulatory role of RAC3 in cisplatin resistance. Cell proliferation was evaluated by colony formation and the IC_{50} values after cisplatin treatment were determined using cell counting kit-8 (CCK-8). The levels of free fatty acids and triglycerides (TGs), as well as the expression of DGAT2 and FASN proteins, were measured to gauge the extent of fatty acid synthesis in cells. Elevated expression of RAC3 was observed in BCa and the cisplatin-resistant BCa cells (T24/DDP). The knockdown of RAC3 within T24/DDP cells was demonstrated to counteract cisplatin resistance. Subsequent analyses identified RAC3 as being notably enriched in the fatty acid synthesis pathway, with Kruppel-like factor 1 (KLF1) emerging as a key upstream regulator. The overexpression of RAC3 was correlated with increased cisplatin resistance in T24/DDP cells, an effect that was mitigated by the addition of the FASN inhibitor, Orlistat. Furthermore, the downregulation of KLF1 suppressed RAC3 expression, disrupted fatty acid synthesis, and attenuated cisplatin resistance in T24/DDP cells. Conversely, the co-overexpression of RAC3 counteracted the effects conferred by KLF1 knockdown. Our study has validated that KLF1 activates RAC3 to mediate fatty acid synthesis and promote cisplatin resistance in BCa, suggesting the KLF1/RAC3 axis as a potential target for combating cisplatin-resistant BCa.

Keywords

bladder cancer, cisplatin resistance, fatty acid synthesis, KLF1, RAC3

Received June 26, 2024; revised September 10, 2024; accepted July 5, 2024

Introduction

Bladder cancer (BCa) is a prevalent malignant neoplasm of the urinary system, severely jeopardizing human health and survival (Lenis et al., 2020). Cisplatin-based chemotherapy is a preferred choice for BCa patients, which is effective in controlling tumor progression and reducing recurrence rates (Stone, 2023). Nonetheless, the onset of acquired cisplatin resistance often threatens the success of treatments (Choi et al., 2014; Kamat et al., 2016). Uncovering the molecular mechanisms of resistance in BCa cells is sure to go a long way toward optimized therapeutic strategies.

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Metabolic reprogramming, the process by which tumor cells adapt to meet their energy demands, has become recognized as a hallmark of cancer, encompassing its onset, advancement, and resistance to treatment (Martínez-Reyes & Chandel, 2021; Pavlova et al., 2022; Stine et al., 2022). Fatty acid metabolism, particularly, is now acknowledged as an essential source of energy for cancer cells, with elevated fatty acid uptake, synthesis, or oxidation being conducive to the proliferation of diverse cancers (Dai et al., 2022; Pham & Park, 2022). Increased fatty acid synthesis is a known contributor to the progression of BCa, and the targeted disruption of this synthesis pathway can inhibit the malignant behaviors of tumor cells (A. Li et al., 2019; Zheng et al., 2016). Modifications in fatty acid synthesis will also impact cancer resistance to therapeutics. For example, an increase in fatty acid synthesis, catalyzed by fatty acid synthase (FASN), has been shown to enhance gemcitabine resistance in pancreatic cancer (Tadros et al., 2017). Similarly, in HER2+ breast cancer (BC), the deubiquitinating enzyme UCHL1 can induce resistance to doxorubicin by promoting the synthesis of free fatty acids (FFA; G. Lu et al., 2021). The specific influence of fatty acid synthesis on cisplatin resistance in BCa is not yet clear.

The small GTPase RAC 3, a member of the Rho GTPase family's Rac subgroup, also known as RAS-related C3 botulinum toxin substrate 3, is involved in critical cellular functions such as cell growth, cytoskeletal reorganization, and the regulation of kinase activity (Lee et al., 2021; Maldonado & Dharmawardhane, 2018; Maldonado et al., 2020). It is generally expressed at low levels in normal tissues (Corbetta et al., 2009; Vaghi et al., 2014), while highly expressed in a range of human cancers, including BCa (Wang et al., 2022), endometrial cancer (EC; Meijuan et al., 2022), and BC (Gest et al., 2013), where it is closely related to tumor progression. Emerging evidence suggests a crucial role for RAC3 in chemoresistance. Specifically, there is a negative correlation between RAC3 expression levels and the sensitivity of lung adenocarcinoma cells to paclitaxel, where the inhibition of RAC3 can reverse paclitaxel resistance (Y. Lu et al., 2024). RAC3 has been identified as a potential therapeutic target in BCa through prognostic modeling (Chen et al., 2024). However, to date, research concerning the role and regulation of RAC3 in the development of cisplatin resistance in BCa is inadequate, necessitating further study.

Through the analysis of signaling pathways and upstream transcription factors associated with RAC3, this work uncovered the significance of RAC3 and the fatty acid synthesis pathway it mediates in the cisplatin resistance of BCa, offering novel insights and potential targets for countering this phenomenon.

Materials and Method

Bioinformatics Analysis

BCa mRNA expression data (normal: 19, tumor: 412) were sourced from The Cancer Genome Atlas (TCGA) database. Using *edgeR*, differential mRNAs were identified between normal and tumor groups with criteria $|\log FC| > 1$ and FDR < 0.05. Target genes were determined by integrating these results with the current literature. The gene set enrichment analysis (GSEA) analysis was performed to identify enriched pathways for these targets. The hTFtarget platform predicted upstream transcription factors for the mRNA of these targets, followed by a Pearson correlation analysis between the genes and factors. Finally, MOLOTOOL was applied to predict the motif binding sites 2,000 bp upstream of the target gene mRNAs.

Cell Culture

Human embryonic kidney-derived 293T cells (CL-0005), the SV-HUC-1 cell line representing immortalized human ureteral epithelium (CL-0222), and the human BCa cell lines RT-112 (CL-0682), HT-1376 (CL-0672), and T24 (CL-0227) were procured from Wuhan Pricella Biotechnology Co., Ltd. (China). The SV-HUC-1 cells were maintained in F-12K complete medium, RT-112 in RPMI-1640 medium, HT-1376 in MEM medium, T24 in McCoy's 5A medium, and 293T in DMEM medium, with each medium being supplemented with 10% fetal bovine serum (Vivacell, USA) and 1% penicillin/streptomycin solution (Dearybio, China). Cultivation was conducted in a humidified incubator set to 37°C and 5% CO_2.

T24 human BCa cells, known for their low cisplatin sensitivity, were used to develop a resistant variant to study cisplatin resistance in BCa (Shi et al., 2022). The cisplatin-resistant cell line, T24/DDP, was constructed through a 6-month exposure to escalating doses of cisplatin (0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 μg/mL; Qin et al., 2021).

Cells were then treated with Orlistat, a FASN inhibitor sourced from Beijing Bio-Lab Technology Co., Ltd. (China), at a concentration of 50 μmol/L for 24 hr prior to further assays (Ye et al., 2023).

Cell Transfection

si-RAC3, oe-RAC3, and si-KLF1, along with their respective negative controls, were procured from

Table 1. Primers for qPCR

Gene	Primer sequence (5'→3')	
RAC3	Forward Primer	TCCCCACCGTTTTTGACAACT
	Reverse Primer	GCACGAACATTCTCGAAGGAG
KLF1	Forward Primer	GAAGAGGACGATGAGAGGGG
	Reverse Primer	ATCCTCCGAACCCAAAAGC
GAPDH	Forward Primer	CTGGGCTACACTGAGCACC
	Reverse Primer	AAGTGGTCGTTGAGGGCAATG

GenePharma (China). Adhering to the provided protocol, these plasmids were introduced into the corresponding cells via transfection using Lipofectamine 2000 (Thermo Fisher Scientific, USA). The cells were then incubated for 48 hr before proceeding with subsequent procedures.

Quantitative Polymerase Chain Reaction

Total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific, USA) and reverse transcribed to cDNA using PrimeScript™ RT Master Mix (TaKaRa, Japan). Quantitative polymerase chain reaction (qPCR) was performed using the SYBR® Green iTaq Master Mix (Bio-Rad, USA) on a CFX96 Real-Time PCR detection system (Bio-Rad), with GAPDH as a reference gene to calculate the relative mRNA expression via the $2^{-\Delta\Delta CT}$ method. Primer details are provided in Table 1.

Colony Formation Assay

In a 12-well plate, 200 cells were seeded per well for incubation at 37°C with 5% CO_2 for 7 to 10 days. The culture medium was discarded, and cells were washed with PBS, fixed with 75% ethanol for 15 min, stained with 1% Crystal violet (Beyotime, China) for 30 min, then rinsed, and dried. Colony counts and photography were facilitated by a Nikon D5600 camera (Nikon, Japan).

Cell Counting Kit-8 Assay

Cells were dispensed into a 96-well plate at 5×10^3 cells per well with fresh medium containing cisplatin at concentrations of 0, 0.5, 1, 2, 4, 8, 16, and 32 μmol/L. After 48 h, 10 μL of cell counting kit-8 (CCK-8) solution (Beyotime, China) was added to each well for a 1-hr incubation. Absorbance was measured at 450 nm using a microplate reader, and IC_{50} values were calculated for each group.

Determination of FFA Concentrations and Triglyceride Levels

Following the protocols outlined by the producers, the cellular levels of FFA were determined utilizing the FFA Quantitative Assay Kit (EFFA-100, BioAssay Systems, USA). Concurrently, the concentration of triglyceride (TG) was evaluated in the cell samples using the TG Quantification Kit (STA-396, Cell Biolabs, USA).

Western Blot

To extract total cellular proteins, RIPA lysis buffer was added to the cells, and protein concentrations were determined using the BCA protein assay kit (Thermo Fisher Scientific, USA). Protein samples were separated by polyacrylamide gel electrophoresis and transferred onto a PVDF membrane for analysis. The membrane was blocked with 5% skim milk for 1 hr before an overnight incubation at 4°C with primary antibodies rabbit anti-human DGAT2 (ab237613), FASN (ab128870) and β-actin (ab8227). After washing with TBST, it was incubated with the secondary goat anti-rabbit IgG antibody for 1 hr at room temperature. The proteins were then visualized using an ECL reagent (Beyotime, China) on a gel imaging system. All antibodies utilized were purchased from Abcam (UK).

Dual-Luciferase Reporter Assay

The wild-type and mutant luciferase reporter vectors, pGL3-RAC3-promoter-WT and pGL3-RAC3-promoter-MUT, were created and then co-transfected with si-NC and si-KLF1 into 293T cells using Lipofectamine 2000 (Thermo Fisher Scientific, USA). Luciferase activity was measured 48 hr later using the dual-luciferase reporter assay system (Promega, USA).

Chromatin Immunoprecipitation

For the chromatin immunoprecipitation (ChIP) analysis, the ChIP Assay Kit (Merck Millipore, Germany) was employed. Cells were crosslinked at 37°C with 1% formaldehyde for 10 min. After PBS washing, cells were lysed with SDS lysis buffer, and DNA was sheared to 200 to 1,000 bp using ultrasonication. The samples were incubated with anti-KLF1 and anti-IgG to precipitate protein-DNA complexes overnight, followed by crosslink reversal. Specific DNA–protein interactions were assessed by qPCR with the primers detailed in Table 2.

Table 2. Primers for ChIP-qPCR

Gene	Primer sequence (5'→3')	
RAC3	Forward Primer	GGACAGGGAGAGCGAGAA
	Reverse Primer	CTGCACGTAAGCCAGGAC

Statistical Analysis

Statistical analysis was conducted with GraphPad Prism 8.0, with all experiments repeated at least three times. Data was shown as $M \pm SD$. The t-test was executed for two-group comparisons, one-way analysis of variance (ANOVA) for multiple groups, and the Pearson correlation for assessing correlations. A $p < .05$ is deemed statistically significant.

Results

The Upregulation of RAC3 in BCa Cells

Our study first compared RAC3 mRNA expression in BCa tumors and adjacent normal tissues via the TCGA database, with tumor tissues showing remarkably higher RAC3 levels (Figure 1A). Further qPCR analysis of RAC3 in the SV-HUC-1 immortalized bladder epithelial cells and three BCa cell lines (RT-112, HT-1376, T24) also revealed increased RAC3 expression in cancer cells (Figure 1B). These findings pointed to the high expression of RAC3 in BCa tissues and cells.

RAC3 Enhances Cisplatin Resistance in BCa Cells

To investigate the role of RAC3 in mediating cisplatin resistance in BCa, we developed a cisplatin-resistant variant of the BCa cell line T24/DDP. The IC_{50} values of T24 and T24/DDP cells were compared using CCK-8, with T24/DDP cells showing a higher IC_{50}, thus indicating successful resistance induction (Figure 2A). RAC3 expression was then quantified in both cell lines by qPCR, where T24/DDP exhibited increased RAC3 levels (Figure 2B). A plasmid for RAC3 knockdown was transfected into T24/DDP. The decrease in RAC3 expression, as measured by qPCR, confirmed the success of transfection (Figure 2C). The colony formation assay was utilized to evaluate the impact of RAC3 knockdown on the proliferative capabilities of T24/DDP cells. It was shown that RAC3 knockdown led to a notable decrease in colony numbers, indicating suppressed cell proliferation (Figure 2D). T24/DDP cells were exposed to a range of cisplatin concentrations (0, 0.5, 1, 2, 4, 8, 16, 32 μmol/L), and the CCK-8 assay was utilized to assess cell viability and to calculate the IC_{50} values. The RAC3 knockdown group exhibited an IC_{50} value of 6.559, which was considerably lower than the IC_{50} value of 12.95 observed in the control group cells (Figure 2E). Collectively, these findings indicated that the knockdown of RAC3 enhances the sensitivity of cisplatin-resistant BCa cells to cisplatin.

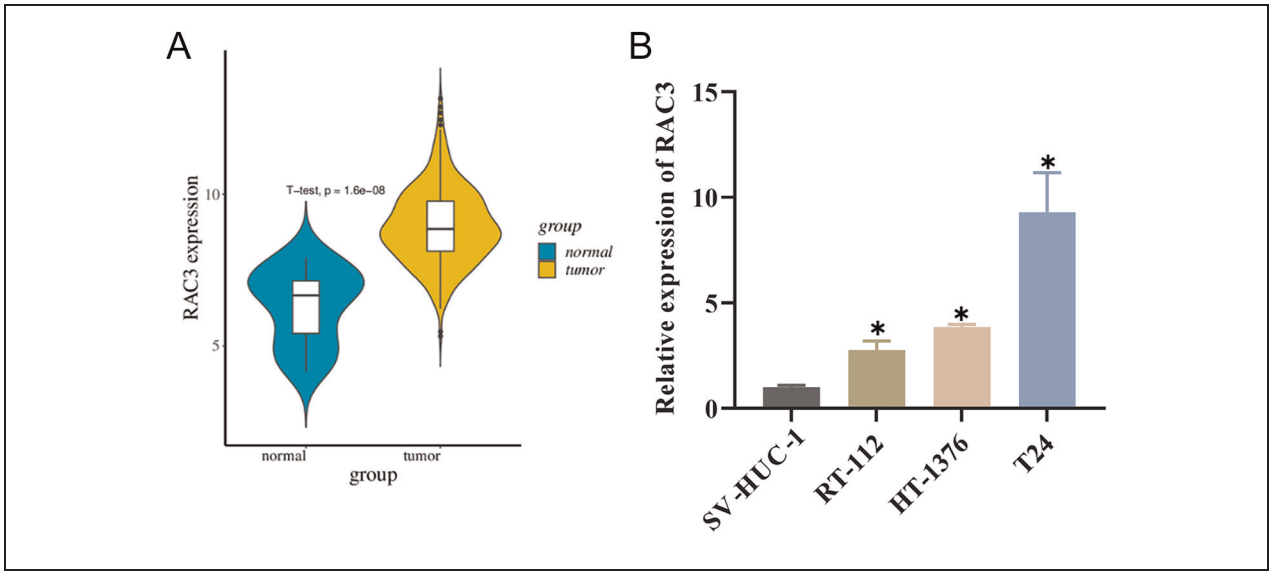

Figure 1. The Expression of RAC3 in BCa

A: mRNA expression levels of RAC3 were obtained from the TCGA database to compare its expression in tumor tissues versus adjacent normal tissues of BCa patients; B: qPCR assessment of RAC3 expression in the immortalized human bladder epithelial cell line SV-HUC-1 and human BCa cell lines RT-112, HT-1376, and T24.

* means $p < .05$.

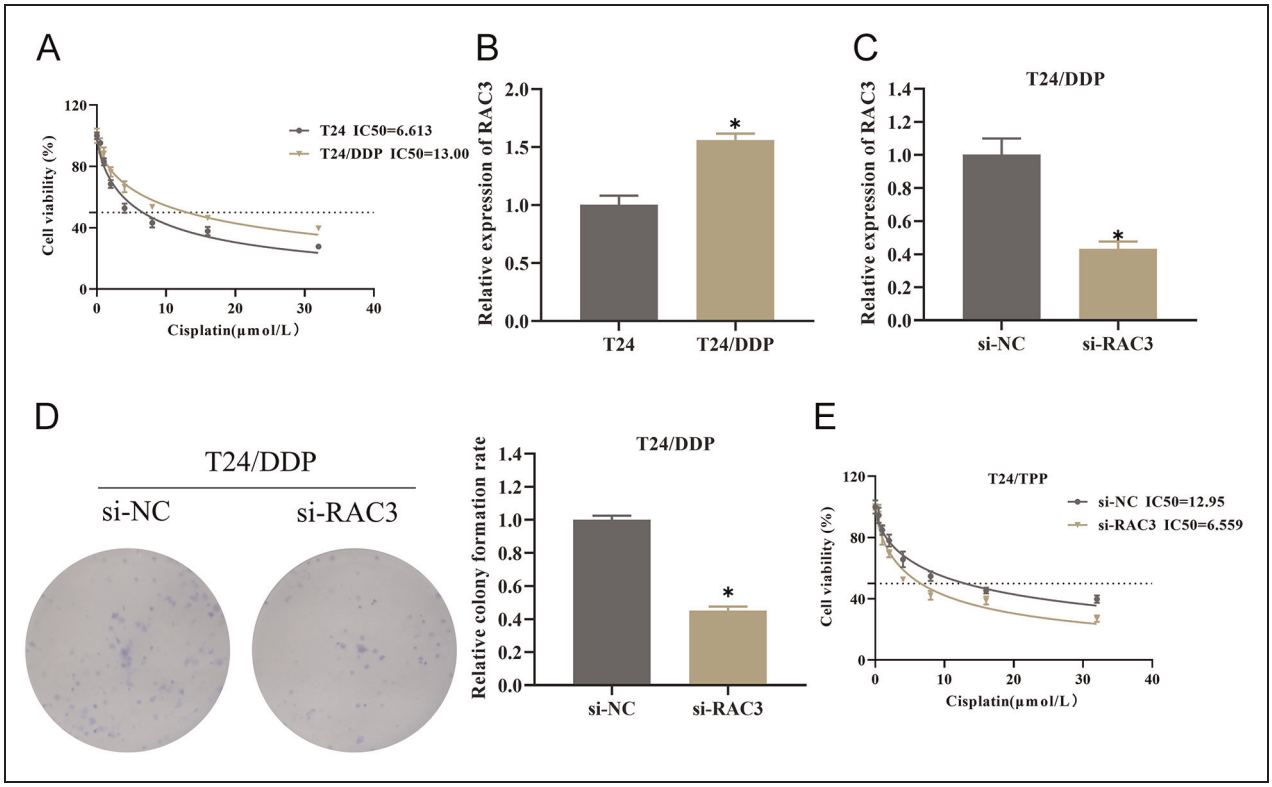

Figure 2. Effects of RAC3 on the Cisplatin Resistance of BCa Cells

A: CCK-8 assay measured the cell viability of T24 and T24/DDP cells and determined the IC_{50} values; B: qPCR examined the expression of RAC3 in cisplatin-resistant BCa cells T24/DDP and the parental T24 cells; C: qPCR assessed the expression levels of RAC3 in T24/DDP cells with si-NC/si-RAC3; D: Colony formation assay assessed the proliferative potential of T24/DDP cells in various groups; E: Following treatment with a range of cisplatin concentrations (0, 0.5, 1, 2, 4, 8, 16, 32 μmol/L), the CCK-8 assay was applied to T24/DDP cells to measure cell viability and to determine the IC_{50} values.

* means $p < .05$.

RAC3 Enhances Cisplatin Resistance in BCa Cells via the Fatty Acid Synthesis Pathway

To further investigate how RAC3 contributes to cisplatin resistance in BCa, we performed GSEA on RAC3, identifying remarkable pathway enrichment in fatty acid synthesis (Figure 3A). A positive correlation between RAC3 and FASN expression was observed (Figure 3B). Analysis of TCGA data showed higher FASN expression in BCa tissues than in adjacent normal tissues (Figure 3C). Thereafter, to substantiate whether RAC3 modulates cisplatin resistance in BCa through the fatty acid synthesis pathway, we established T24/DDP cell line groups treated with oe-NC + DMSO, oe-RAC3 + DMSO, and oe-RAC3 + Orlistat (FASN inhibitor). We then quantified the levels of FFA within the distinct groups of T24/DDP cells. The data demonstrated that overexpression of RAC3 elevated FFA levels, but the introduction of Orlistat normalized them to the control group levels (Figure 3D). In addition, overexpression of RAC3 also heightened TG levels in T24/DDP cells, and the combined treatment with Orlistat lessened this impact (Figure 3E). Western blot (WB) was conducted to explore the expression levels of DGAT2 and FASN proteins in T24/DDP cells. The results demonstrated that the overexpression of RAC3 led to a notable increase in the expression of both DGAT2 and FASN. Nonetheless, the co-administration of Orlistat with RAC3 overexpression resulted in the protein expression levels returning to the control levels (Figure 3F). Furthermore, the colony formation assay revealed that RAC3 overexpression enhanced the proliferative ability of T24/DDP cells, and the joint application of Orlistat diminished the proliferative effect induced by RAC3 overexpression (Figure 3G). Ultimately, T24/DDP cells were treated with cisplatin at concentrations of 0, 0.5, 1, 2, 4, 8, 16, and 32 μmol/L, and the CCK-8 assay measured cell viability and IC_{50} values across groups. In the T24/DDP cells where RAC3 was overexpressed, the IC_{50} value reached 21.96, which was considerably higher than

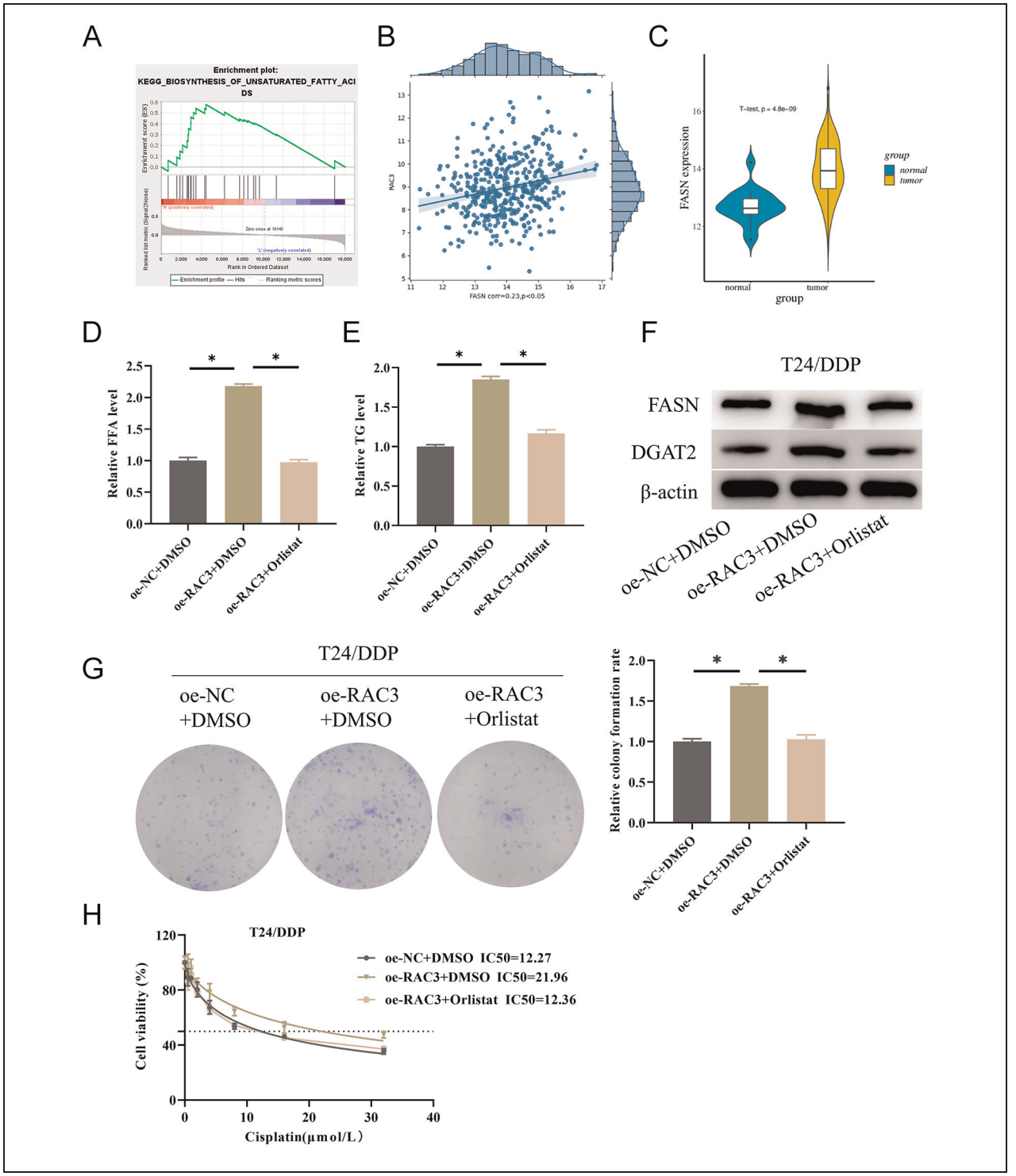

Figure 3. Impact of RAC3 in Fatty Acid Synthesis and Cisplatin Resistance in BCa Cells

A: GSEA analysis of RAC3-enriched pathways; B: Pearson correlation of RAC3 and FASN expression; C: TCGA database analysis of FASN expression in BCa tissues; D: FFA level measurement in T24/DDP cell groups treated with oe-NC+DMSO, oe-RAC3+DMSO, and oe-RAC3+Orlistat; E: TG level detection in T24/DDP cells; F: WB detection of DGAT2 and FASN expression in T24/DDP cells; G: Colony formation assay for T24/DDP cell proliferation; H: CCK-8 assay for cell viability and IC_{50} analysis in T24/DDP cells treated with cisplatin concentrations (0, 0.5, 1, 2, 4, 8, 16, 32 μmol/L).

* means $p < .05$.

the control group's IC_{50} value of 12.27. Nonetheless, the Orlistat administration brought it down to 12.36, nearly identical to the control group (Figure 3H). The data demonstrated that RAC3 facilitated cisplatin resistance in BCa cells via the upregulation of fatty acid synthesis.

KLF1 Transcriptionally Activates RAC3

To delve into the molecular mechanisms involved in RAC3 regulation of cisplatin resistance in BCa, we employed hTFtarget to identify the transcription factor kruppel-like factor 1 (KLF1) within 2,000 bp upstream of the RAC3 promoter. Pearson correlation analysis demonstrated a significant positive correlation between RAC3 and KLF1 expression levels (Figure 4A and 4B). Moreover, the KLF1 binding site on the RAC3 promoter was predicted to be located at 1691 to 1704 bp using MOLOTOOL, identified as the sequence GGGGCGGGTCCCCG (Figure 4C). We then examined the expression levels of KLF1 in BCa patient tumor tissues and adjacent normal tissues using data from the TCGA database, revealing that KLF1 expression was markedly elevated in tumor tissues relative to adjacent normal tissues (Figure 4D). qPCR detected that, in comparison with the human bladder epithelial immortalized cell line SV-HUC-1, the expression levels of KLF1 were also elevated in the BCa cell lines RT-112, HT-1376, and T24 (Figure 4E). Dual-luciferase and ChIP experiments validated the binding of KLF1 to RAC3, where si-KLF1 significantly lowered the luciferase activity of RAC3-WT but exerted no influence on RAC3-MUT (Figure 4F). Moreover, the application of anti-KLF1 resulted in a pronounced elevation in RAC3 enrichment (Figure 4G). These collective results posited that KLF1 served as an upstream regulator of RAC3 transcription.

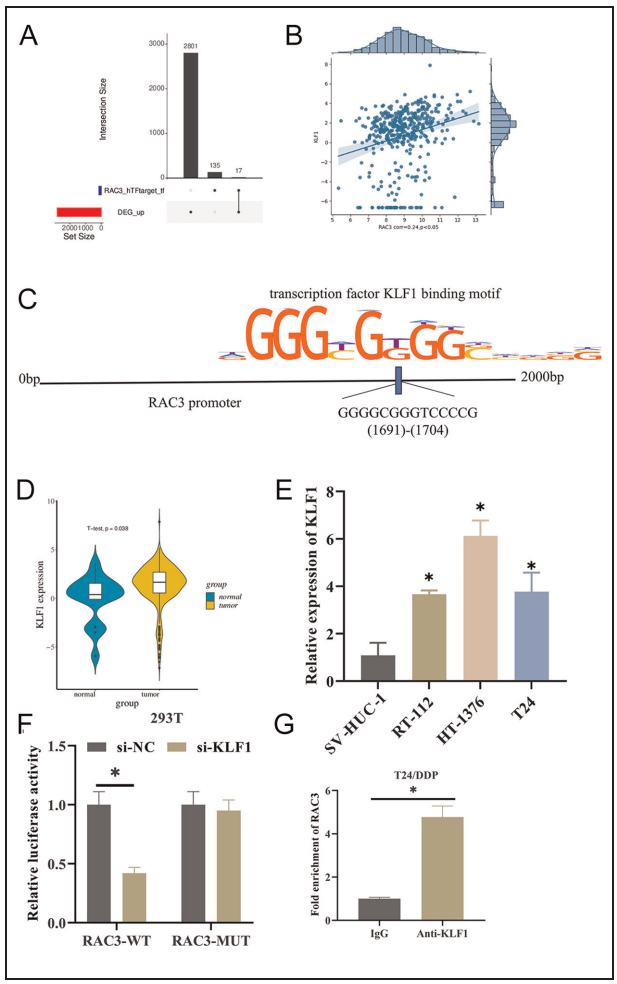

Figure 4. KLF1 Functions as the Upstream Regulatory Molecule of RAC3

A: Upset plot predicting potential upstream target genes and differentially expressed genes for RAC3; B: Pearson analysis of RAC3 and KLF1 correlation; C: Identification of potential binding sites of KLF1 on the upstream of RAC3 promoter; D: TCGA analysis of KLF1 expression in BCa tissues and normal adjacent cancerous tissue; E: qPCR detection of KLF1 expression in the human bladder epithelial immortalized cell line SV-HUC-1 and human BCa cells RT-112, HT-1376, T24; F-G: Dual-luciferase and ChIP experiments determined the binding relationship between KLF1 and RAC3.

* means $p < .05$.

KLF1 Activates RAC3 to Mediate the Fatty Acid Synthesis Pathway and Enhance Cisplatin Resistance in BCa

To confirm the hypothesis that KLF1 may transcriptionally activate RAC3, thereby mediating the fatty acid synthesis pathway and promoting cisplatin resistance in BCa, we created T24/DDP cell groups treated with si-NC + oe-NC, si-KLF1 + oe-NC, and si-KLF1 + oe-RAC3. qPCR aided us in quantifying RAC3 expression levels across the groups, uncovering that KLF1 knockdown diminished RAC3 expression, an effect that was mitigated by the co-overexpression of RAC3 (Figure 5A). The colony formation assay revealed that KLF1 knockdown curbed the proliferation ability of T24/DDP cells, which was counteracted by RAC3 overexpression (Figure 5B and 5C). We assessed fatty acid synthesis by measuring the levels of FFA and TGs in the various cell groups. The data showed that KLF1 knockdown markedly decreased the levels of these lipids, and the co-overexpression of RAC3 restored the levels to the control levels (Figure 5D and 5E). WB results showed that KLF1 knockdown decreased the protein expression levels of DGAT2 and FASN in T24/DDP cells, but RAC3

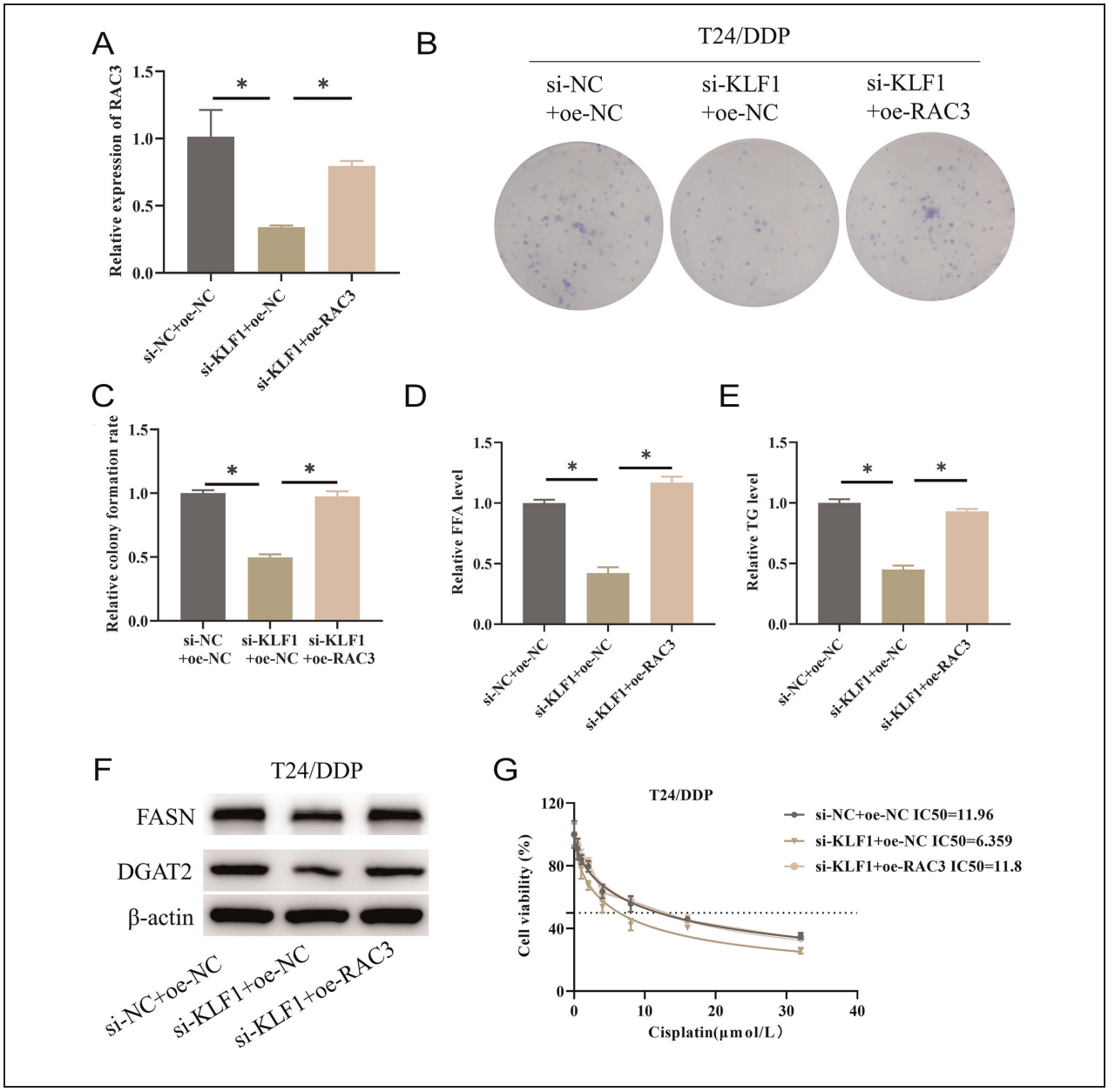

Figure 5. KLF1 Activates RAC3 to Mediate the Fatty Acid Synthesis Pathway and Enhance Cisplatin Resistance in BCa
A: T24/DDP cell groups with si-NC+oe-NC, si-KLF1+oe-NC, si-KLF1+oe-RAC3 were constructed. qPCR measured RAC3 expression across various groups; B-C: Colony formation assay assessed T24/DDP cell proliferation; D: FFA levels in T24/DDP cells were determined; E: TG levels in T24/DDP cells were measured; F: WB detected DGAT2 and FASN expression in T24/DDP cells; G: T24/DDP cells were treated with a gradient of cisplatin concentrations (0, 0.5, 1, 2, 4, 8, 16, 32 μmol/L). The CCK-8 assay was executed to assess cell viability and calculate IC_{50} values.
* means $p < .05$.

overexpression negated this effect (Figure 5F). Thereafter, T24/DDP cells were subjected to treatment with a gradient of cisplatin concentrations, and the CCK-8 assay was employed to measure cell viability and to calculate the IC_{50} values. The IC_{50} value for the KLF1 knockdown group was identified as 6.359, which was considerably lower than 11.95 in the control group. Notably, the co-overexpression of RAC3 with KLF1 knockdown restored the IC_{50} value to a level similar to the control group (Figure 5G). These findings demonstrated that KLF1 could transcriptionally activate RAC3, thereby facilitating fatty acid synthesis and augmenting cisplatin resistance in BCa.

Discussion

Patients with BCa frequently encounter cisplatin resistance, which can lead to the failure of chemotherapy treatments. Therefore, it is imperative to seek out strategies effective in mitigating cisplatin resistance in BCa (Kamat et al., 2016; Powles et al., 2020). We elucidated the mechanisms by which RAC3 influences cisplatin resistance in BCa, wnich was that RAC3 could be activated by the upstream transcription factor KLF1, leading to an increase in its expression, which in turn induced the fatty acid synthesis pathway and resulted in enhanced cisplatin resistance in BCa cells. This highlighted the crucial role of RAC3 in the development of cisplatin resistance in BCa.

Extant literature has established that RAC3 is implicated in the malignant behaviors of cancer cells, such as cell proliferation, migration, invasion, apoptosis, and autophagy, and is considered to play a pivotal role as an oncogene in cancer progression (Cheng et al., 2020; Rubio et al., 2017). Anomalous overexpression of RAC3 has been found in various human solid tumors, including BCa (Cheng et al., 2020; P. Huang et al., 2023). The present study also identified a pronounced elevation in RAC3 expression within BCa tissues. Furthermore, it has been posited that the expression levels of RAC3 may modulate tumor sensitivity to chemotherapeutic agents (Rubio et al., 2017). Specifically, Cai et al. (2024) found that RAC3 is linked to gemcitabine resistance in BCa, with higher expression in resistant patients, and that RAC3 knockdown increases cell sensitivity to gemcitabine. Despite this evidence linking RAC3 to chemoresistance in cancer, its contribution to cisplatin resistance in BCa has not been elucidated. To this end, we established a cisplatin-resistant BCa cell line, T24/DDP, to examine the effects of RAC3 on cisplatin resistance in BCa. We found that RAC3 was upregulated in cisplatin-resistant BCa cells, and the knockdown of RAC3 expression diminished the cisplatin resistance of these cells. In essence, RAC3 may serve as a potential biomarker of cisplatin resistance and a therapeutic target in BCa.

Subsequently, GSEA helped us identify that RAC3 was notably enriched in pathways associated with fatty acid synthesis. Tumor cells often enhance the biosynthesis of fatty acids to fuel their vigorous proliferation, and this metabolic dysregulation significantly feeds into the development and progression of tumors (Chao et al., 2019; Jin et al., 2021). FASN, an enzyme pivotal to fatty acid synthesis, exhibits expression levels that are intimately associated with the malignant progression of tumors (Yan et al., 2019). RAC3 has been shown to stimulate cancer cell proliferation and invasiveness in EC by upregulating FASN expression (Meijuan et al., 2022), which corroborates our findings that RAC3 facilitated fatty acid synthesis in BCa. Furthermore, it has been established that cisplatin-resistant cancer cells often undergo a reprogramming of fatty acid metabolism (Tan et al., 2022). Increased fatty acid synthesis can contribute to chemotherapy resistance in tumors (Yamamoto et al., 2024). Therefore, the mechanism through which RAC3 enhances cisplatin resistance in BCa may be linked to the activation of the fatty acid synthetic pathway. To validate this, we performed further experiments with the FASN inhibitor, Orlistat. We discovered that overexpression of RAC3 can activate the fatty acid synthesis pathway to enhance cisplatin resistance in BCa, and the administration of Orlistat attenuated the impact of RAC3 overexpression, highlighting that targeting RAC3 to regulate fatty acid synthesis could be a promising strategy to address cisplatin resistance in BCa.

Using correlation analysis to predict potential upstream transcription factors that might regulate RAC3, we identified KLF1 as a transcription factor to further explore the mechanisms of RAC3 in regulating cisplatin resistance in BCa. KLF1 is a conserved zinc-finger transcription factor that can change the oncogenic properties by activating the expression of genes downstream. For example, in cervical cancer, KLF1 promotes tumor progression by interfering with the proliferation- and metastasis-related factors (Zhu et al., 2018). There have been no reports on the role of KLF1 in BCa until this study. Our current investigation revealed that KLF1 was overexpressed in BCa, similar to results from studies on gastric (S. Li et al., 2021) and colorectal cancers (Z. Huang et al., 2022). We also illuminated that KLF1 was capable of transcriptionally activating the RAC3-mediated fatty acid synthesis pathway, which contributed to cisplatin resistance in BCa.

In brief, our findings present a fresh insight that the KLF1/RAC3 axis, through the promotion of fatty acid synthesis, may strengthen cisplatin resistance in BCa, potentially providing a promising strategy for the clinical reduction of cisplatin resistance in BCa patients. However, the limitation of this study is that it was performed only at the cellular level and lacked validation of animal models and detection of clinical samples. In the future, additional in vivo experiments are necessary to confirm the mechanisms by which RAC3 contributes to cisplatin resistance in BCa.

Author Contributions

LS contributed to the study design. QX conducted the literature search. RC acquired the data. JZ wrote the article. WS

performed data analysis and drafted. QX revised the article. LS gave the final approval of the version to be submitted.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The project of Shaoxing City Science and Technology plan in 2023 (No. 2023A14040).

Ethics Approval and Consent to Participate

Ethical approval is not required for this study in accordance with local or national guidelines.

Consent to Participate Statement

Patient consent was not required in accordance with local or national guidelines.

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Data Availability Statement

The data and materials in the current study are available from the corresponding author on reasonable request.

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