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Circ-FABP5 activates the Wnt/β-catenin signaling pathway and mediates radiation-induced fibrotic response in mouse bladder fibroblast cell line via the miR-17-5p/HIF1A/CTNNB1 axis

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► ***Original article***

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ABSTRACT

Background: Age-related bladder fibrosis (BF) is a prevalent pathological condition in the elderly, exacerbated by radiotherapy for pelvic malignancies, leading to severe bladder dysfunction. This study aimed to determine whether Circular Fatty Acid Binding Protein 5 (Circ-FABP5) activates the Wnt/β-Catenin signaling pathway to mediate radiation-induced fibrotic changes in a mouse bladder fibroblast cell line (CP-M066) through the miR-17-5p/HIF1A/CTNNB1 axis. ***Materials and Methods:*** Mouse bladder fibroblast cell line (CP-M066) was treated with varying doses of radiation (0–8 Gy) using a Shanghai Med-X Linear Accelerator and cultured with transforming growth factor (TGF)-β1 to induce fibrosis. Cell viability, apoptosis, and protein expression were assessed using MTT assay, flow cytometry, and Western blot (WB). qRT-PCR quantified miR-17-5p, HIF1A, and CTNNB1 expression. Dual-luciferase assays confirmed miR-17-5p targeting of CTNNB1. All radiation doses (0, 2, 4, 6 and 8 Gy) were evaluated to assess dose-dependent cellular and molecular responses. ***Results:*** Circ-FABP5 overexpression reduced fibroblast viability ($P<0.05$) and increased apoptosis ($P<0.01$) after 4 Gy radiation exposure. miR-17-5p expression increased by 2.3-fold, while HIF1A and CTNNB1 decreased by 60% and 55%, respectively ($P<0.05$). Silencing CTNNB1 inhibited fibroblast proliferation by 45% ($P<0.01$). Dual-luciferase assays confirmed miR-17-5p binding to CTNNB1, reducing luciferase activity by 50% ($P<0.05$). Radiation significantly enhanced fibrosis markers (α-SMA, collagen I) by 70% ($P<0.01$), which were partially reversed by Circ-FABP5 modulation. ***Conclusion:*** Circ-FABP5 regulates radiation-induced fibrotic responses in mouse bladder fibroblast cells by activating the Wnt/β-Catenin signaling pathway via the miR-17-5p/HIF1A/CTNNB1 axis. These findings provide potential molecular targets for mitigating radiation-induced bladder dysfunction.

INTRODUCTION

Bladder fibrosis (BF) is a debilitating pathological condition characterized by excessive extracellular matrix (ECM) deposition, leading to progressive structural and functional deterioration of the bladder, particularly in elderly individuals [1]. Radiotherapy (RT), a cornerstone treatment for pelvic malignancies such as prostate, cervical, and rectal cancers, is one of the major contributors to BF due to its ability to induce chronic inflammation, oxidative stress, and tissue remodeling [2]. Radiation-induced bladder injury (RIBI) manifests as fibrotic thickening, reduced bladder compliance, and impaired urine storage and voiding function, which together severely impact patients' quality of life [3]. However, the precise molecular mechanisms underlying RIBI and age-related BF remain poorly understood, highlighting the need for further mechanistic investigations to identify effective therapeutic targets.

Circular RNAs (circRNAs) are a class of non-coding RNAs characterized by covalently closed-loop structures that confer high stability and regulatory potential. These molecules modulate gene expression through interactions with microRNAs (miRNAs) and proteins, influencing biological processes such as cell differentiation, apoptosis, and fibrosis [4]. Among them, Circular Fatty Acid Binding Protein 5 (Circ-FABP5) has been shown to regulate lipid metabolism, signal transduction, and proliferation in multiple tissues [5]. Fatty Acid Binding Protein 5 (FABP5), the linear isoform of Circ-FABP5, plays crucial roles in tumor progression, lipid metabolic reprogramming, and radiation response in several cancers, including bladder and cervical cancer [6-8].

The Wnt/β-catenin signaling pathway, a key regulator of cellular proliferation and fibrotic remodeling, is frequently activated in tissue fibrosis and modulated by circRNAs [9]. Hypoxia-inducible factor-1α (HIF1A) and β-catenin (CTNNB1) act as central mediators of this pathway, coordinating fibroblast activation and ECM accumulation [10-13].

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MicroRNA-17-5p (miR-17-5p) has been identified as a regulator of fibrosis through suppression of CTNNB1, suggesting a potential miR-17-5p/HIF1A/CTNNB1 signaling axis in BF [14, 15].

Radiation-induced BF is particularly relevant in elderly populations where pelvic RT is frequently administered. Ionizing radiation produces reactive oxygen species (ROS), leading to DNA damage, fibroblast activation, and ECM accumulation [16, 17]. Studies have shown that radiation doses of 2-8 Gy, similar to those used in clinical practice, induce significant fibrotic remodeling and late bladder toxicity [18–20]. Despite this, the interaction between Circ-FABP5, miR-17-5p, and Wnt/β-catenin signaling under radiation stress remains largely unexplored.

Therefore, this study aimed to elucidate the role of Circ-FABP5 in radiation-induced BF through the miR-17-5p/HIF1A/CTNNB1 axis using an in vitro model of mouse bladder fibroblast cells (CP-M066). This work provides new mechanistic insight into the molecular interplay between circRNA signaling and radiation response, potentially identifying novel therapeutic targets for preventing bladder dysfunction in irradiated patients.

MATERIALS AND METHODS

Research materials

Mouse bladder fibroblast cell line (CP-M066, Shanghai Chunmai Biotechnology Co., Ltd., China) was used to establish an age-related bladder fibrosis (BF) model. Cells were authenticated by short tandem repeat (STR) analysis and confirmed free of mycoplasma contamination using the MycoAlert™ Mycoplasma Detection Kit (Lonza, Switzerland). Transforming growth factor-β1 (TGF-β1; P1230, OK Biotech Co., Ltd., China) was used to induce fibrosis. Circular Fatty Acid Binding Protein 5 (Circ-FABP5) was expressed using the pcDNA3.1 vector (Invitrogen, USA) with an N-terminal His tag (NTCC, China). Radiation treatment was administered using a Shanghai Med-X Linear Accelerator (Shanghai Medical Instruments Co., Ltd., China) at doses of 0, 2, 4, 6, and 8 Gy.

Cell selection criteria: Cells were selected based on verified mouse (Mus musculus) origin, viability > 90%, normal fibroblast morphology under microscopy, and passage number < 10 to avoid phenotypic drift. Exclusion criteria included mycoplasma contamination, morphological abnormalities (e.g., granularity, detachment), or poor reproducibility in preliminary assays.

Cell culture and cryopreservation

CP-M066 cells were cultured in T25 flasks (Corning, USA) sterilized with 75% ethanol and maintained at 37 °C in 5% CO_2 (Boxun Medical Equipment Co., Ltd., China). The growth medium consisted of Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; ExCell Bio, China) and 1% penicillin/streptomycin (P/S; Beyotime Biotechnology, China). Cells were sub-cultured at 85% confluence using 0.25% Trypsin-EDTA (Sangon Biotech, China) and seeded at a 1:3 ratio.

For cryopreservation, cells were suspended in freezing medium (90% FBS + 10% DMSO; Sigma-Aldrich, USA), aliquoted at 5×10^6–1×10^7 cells/mL, and stored at −80 °C for 24 h before transfer to liquid nitrogen (Yuyan Instruments Co., Ltd., China). Culture surfaces were coated with 0.1% gelatin (Macklin Biochemical, China) to promote adhesion and viability.

Radiation treatment

Cells were irradiated using a 6 MV photon beam from the Shanghai Med-X Linear Accelerator, with a dose rate of 200 cGy/min. Radiation doses of 0, 2, 4, 6 and 8 Gy were delivered in a single fraction to simulate clinical pelvic RT exposure. Dosimetry calibration was verified using a PTW Ionization Chamber (PTW, Germany). Following irradiation, cells were incubated for 48 hours before further analyses. All dose points (0–8 Gy) were evaluated in MTT, apoptosis, and gene expression assays to assess dose-response relationships.

Cell transfection and grouping

Cells in the logarithmic growth phase were seeded at 5×10^5 cells/well in 6-well plates (Corning, USA). Transfections were performed with Lipofectamine 2000 (Invitrogen, USA) using the following constructs: miR-17-5p mimics, anti-miR-17-5p, si-HIF1A, si-CTNNB1, and pcDNA3.1-Circ-FABP5 (all from GenePharma Co., Ltd., China). Experimental groups included: control, 2.0 mM FABP5, miR-NC, miR-17-5p overexpression, anti-NC, anti-miR-17-5p, si-NC, si-HIF1A, si-CTNNB1, FABP5 + anti-NC, FABP5 + miR-17-5p, FABP5 + si-HIF1A, and FABP5 + pcDNA3.1-CTNNB1. After 6 hours of transfection, cells were refreshed with complete DMEM, exposed to 4 Gy radiation, and harvested after 48 hours for analysis.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated using the TRIzol™ Reagent Kit (Sangon Biotech, China) and quantified with a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA). Reverse transcription was performed using the PrimeScript™ RT Reagent Kit (Takara Bio, Japan). qRT-PCR was carried out on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA) using SYBR Green Master Mix (Roche Diagnostics, Germany).

Relative mRNA expression was normalized to GAPDH, whereas miRNA expression was normalized to the U6 small nuclear RNA (U6 snRNA), which

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served as the internal reference gene for miR-17-5p quantification (table 1). The $2^{-\Delta\Delta Ct}$ method was used to calculate fold changes in gene expression. All reactions were performed in triplicate (n = 3).

Table 1. Primer sequences used for qRT-PCR.

Name	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
miR-17-5p	CAAAGUGCUUACAGUG-CAGGUAG	CUACCUGCACUGU-AAGCACUUUG
HIF1A	TAGCCGAGGAAGAAC-TATGAAC	CTGAGGTT-GGTTACTGTTGGTA
CTNNB1	AGGGCCGGACUCGUCAT-ACU	GGCGCAACAC-CATGTACCC
GAPDH	AAGAAGGTGGTGAA-GCAGGC	TCCACCACCCTGTT-GCTGTA
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTT-GCGT

MTT assay

Cell viability was measured using the MTT Assay Kit (Beyotime Biotechnology, China). Cells were seeded in 96-well plates (5×10^3 cells/well) and exposed to 0–8 Gy radiation and 0-3.5 mM FABP5. After 48 hours, 20 µL MTT reagent was added, followed by 150 µL DMSO (Sigma-Aldrich, USA) for formazan solubilization. Absorbance at 490 nm was recorded using a BioTek ELx808 Microplate Reader (BioTek Instruments, USA). Data represent mean ± SD of three independent experiments (n = 3).

Flow cytometry

Apoptosis was analyzed using the Annexin V-FITC/PI Apoptosis Detection Kit (Beyotime Biotechnology, China). Cells (1×10^6 cells/mL) were washed twice with PBS, stained with 5 µL Annexin V-FITC and 5 µL propidium iodide (PI), and analyzed within one hour using a BD FACSCalibur™ Flow Cytometer (BD Biosciences, USA). Results were expressed as percentages of early + late apoptotic cells.

Western blotting

Proteins were extracted with RIPA Lysis Buffer (Beyotime Biotechnology, China) containing protease inhibitor cocktail (Roche Diagnostics, Germany). Protein concentrations were determined using the BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Equal amounts (30 µg) of protein were separated on 10% SDS-PAGE gels (Sangon Biotech, China) and transferred to PVDF membranes (Merck Millipore, Germany).

Membranes were incubated overnight at 4 °C with primary antibodies: anti-Bax, anti-Bcl-2, anti-α-SMA, anti-collagen I, and anti-GAPDH (Abcam, UK), each at 1:500-1:10 000 dilutions, followed by HRP-conjugated secondary antibodies (Santa Cruz Biotechnology, USA; 1:4000). Bands were visualized using an ECL Detection Kit (Thermo Fisher Scientific, USA) and quantified with a Tanon 5200 Imaging System (Tanon Science & Technology, China).

Dual-luciferase reporter assay

The 3′UTR sequences of HIF1A and CTNNB1 were cloned into pGL3-Promoter vectors (Promega, USA). CP-M066 cells were co-transfected with 0.12 µg luciferase constructs and 40 nmol/L miR-17-5p mimics using Lipofectamine 2000 (Invitrogen, USA). Forty-eight hours after transfection, luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega, USA) on a GloMax® Luminometer (Promega, USA). Renilla luciferase served as the internal control for normalization.

Data analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., USA). Results are expressed as mean ± standard deviation (SD). Normality was confirmed by the Shapiro–Wilk test, and comparisons among groups were performed using one-way ANOVA followed by least significant difference (LSD) post-hoc tests. A $P<0.05$ was considered statistically significant. All experiments were repeated independently at least three times (n = 3).

RESULTS

Radiation and FABP5 effects on cell viability and apoptosis

To investigate the effects of radiation and Circular Fatty Acid Binding Protein 5 (Circ-FABP5) on mouse bladder fibroblast (CP-M066) viability and apoptosis, we first examined cell survival using the MTT assay across radiation doses ranging from 0 to 8 Gy administered via the Shanghai Med-X Linear Accelerator. A clear dose-dependent decrease in cell viability was observed. Specifically, viability decreased to 85% ± 4.3 at 2 Gy ($P < 0.05$), 65% ± 4.8 at 4 Gy ($P<0.01$), 50% ± 3.9 at 6 Gy ($P<0.001$), and 35% ± 3.1 at 8 Gy ($P<0.001$) compared with controls (0 Gy, 100% ± 5.2). These findings demonstrate the radiosensitivity of CP-M066 fibroblasts and the progressive cytotoxicity with increasing radiation doses. Based on the dose–response curve, 4 Gy was selected as the optimal dose for subsequent mechanistic experiments, as it provided a consistent fibrotic response without excessive lethality.

Next, we evaluated the combined effect of 2.0 mM FABP5 treatment and 4 Gy irradiation, a combination selected from preliminary optimization (0.5-3.5 mM). Cell viability in the combined group declined to 40% ± 3.5 of control ($P < 0.01$ vs. control; $P<0.05$ vs. radiation alone), suggesting a synergistic cytotoxic effect. Annexin V-FITC/PI flow cytometry revealed a corresponding increase in apoptosis: 5.1% ± 0.8 (control), 15.3% ± 1.2 (radiation alone, $P < 0.05$), 12.8% ± 1.0 (FABP5 alone, $P<0.05$), and 35.2% ± 2.1 (radiation + FABP5, $P<0.01$). These results confirm that FABP5 enhances radiation-induced programmed cell death.

Supporting these findings, Western blot analysis showed an increase in pro-apoptotic Bax and a

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decrease in anti-apoptotic Bcl-2 protein expression. In the radiation + FABP5 group, Bax increased 1.5 ± 0.12-fold (P<0.05), while Bcl-2 decreased to 0.55 ± 0.08-fold (P<0.05) relative to GAPDH, indicating a shift toward apoptosis. All results were obtained 48 hours post-radiation, allowing for comparative evaluation before (0 Gy) and after (2-8 Gy) exposure. Data are summarized in table 2, with corresponding visuals in figures 1 and 2.

Table 2. Effect of radiation and FABP5 on CP-M066 cell viability and apoptosis.

Group	Radiation Dose (Gy)	FABP5 (mM)	Viability (% of Control)	Apoptosis Rate (%)
Control	0	0	100 ± 5.2	5.1 ± 0.8
2 Gy	2	0	85 ± 4.3*	10.5 ± 0.9*
4 Gy	4	0	65 ± 4.8*	15.3 ± 1.2*
6 Gy	6	0	50 ± 3.9*	22.7 ± 1.8**
8 Gy	8	0	35 ± 3.1*	29.8 ± 2.2**
FABP5 Only	0	2.0	70 ± 5.0*	12.8 ± 1.0*
Radiation + FABP5	4	2.0	40 ± 3.5*	35.2 ± 2.1*

*Values are mean ± SD from three independent experiments (n = 3). *P < 0.05, *P < 0.01 vs. control (one-way ANOVA, LSD post-hoc test). FABP5, Fatty Acid Binding Protein 5; Gy, Gray; SD, standard deviation.

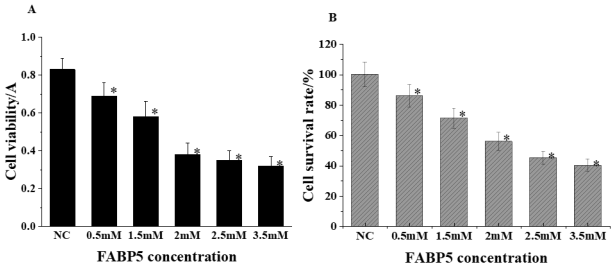

Figure 1. Effects of radiation and FABP5 on CP-M066 cell viability and apoptosis. **(A)** MTT assay results showing radiation dose–dependent reduction in viability (0–8 Gy). **(B)** Representative flow cytometry scatter plots showing viable, early apoptotic, late apoptotic, and necrotic populations. Error bars: SD; asterisks: significance levels as in table 2.

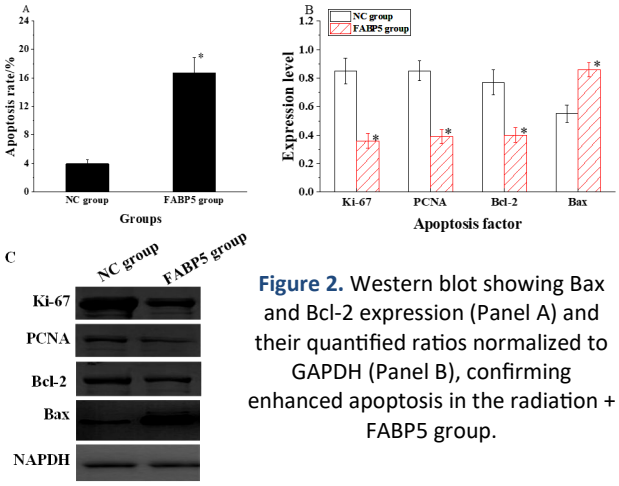

Figure 2. Western blot showing Bax and Bcl-2 expression (Panel A) and their quantified ratios normalized to GAPDH (Panel B), confirming enhanced apoptosis in the radiation + FABP5 group.

Fibrosis marker expression before and after radiation

Radiation exposure is a well-known inducer of fibrosis. To confirm this, we analyzed α-smooth muscle actin (α-SMA) and collagen I expression by Western blot in fibroblasts exposed to 0–8 Gy radiation. A dose-dependent increase in both fibrosis markers was observed. At 2 Gy, α-SMA and collagen I increased to 1.3 ± 0.1-fold and 1.25 ± 0.1-fold (P < 0.05). At 4 Gy, α-SMA rose to 1.7 ± 0.2-fold and collagen I to 1.65 ± 0.15-fold (P<0.01). Further increases were noted at 6 Gy (2.0 ± 0.2) and 8 Gy (2.4 ± 0.3).

Treatment with FABP5 alone (2.0 mM) significantly reduced both α-SMA and collagen I expression to 0.6 ± 0.1 and 0.65 ± 0.1 (P<0.05), indicating an anti-fibrotic role. In the radiation + FABP5 group, marker levels returned close to baseline (1.0 ± 0.15 for α-SMA, 1.0 ± 0.1 for collagen I; P>0.05 vs. control), demonstrating that FABP5 mitigates radiation-induced fibrotic activation. Results before and after radiation are summarized in table 3.

Table 3. Fibrosis marker expression before and after radiation.

Group	Radiation Dose (Gy)	α-SMA (Fold Change)	Collagen I (Fold Change)
Control	0	1.0 ± 0.1	1.0 ± 0.1
2 Gy	2	1.3 ± 0.1*	1.25 ± 0.1*
4 Gy	4	1.7 ± 0.2*	1.65 ± 0.15*
6 Gy	6	2.0 ± 0.2*	2.05 ± 0.2*
8 Gy	8	2.4 ± 0.3*	2.35 ± 0.25*
FABP5 Only	0	0.6 ± 0.1*	0.65 ± 0.1*
Radiation + FABP5	4	1.0 ± 0.15	1.0 ± 0.1

*Data represent mean ± SD (n = 3). *P < 0.05, *P < 0.01 vs. control. α-SMA, alpha-smooth muscle actin; ECM, extracellular matrix; FABP5, Fatty Acid Binding Protein 5.

Circ-FABP5 modulates the miR-17-5p/HIF1A/CTNNB1 axis

Overexpression of Circ-FABP5 via pcDNA3.1 vector in irradiated fibroblasts (4 Gy) significantly altered the Wnt/β-catenin signaling components. qRT-PCR revealed that miR-17-5p expression increased to 2.3 ± 0.2-fold (P<0.01), while HIF1A and CTNNB1 mRNA levels decreased to 0.4 ± 0.05-fold and 0.45 ± 0.06-fold of control (P<0.05), respectively. These results suggest that Circ-FABP5 upregulates miR-17-5p, which subsequently downregulates HIF1A and CTNNB1.

Functional validation using siRNA-mediated CTNNB1 silencing reduced fibroblast proliferation by 45% ± 3.5 (P<0.01 vs. si-NC), confirming CTNNB1's role in fibroblast viability. Notably, non-irradiated Circ-FABP5-overexpressing cells exhibited only a modest miR-17-5p increase (1.5 ± 0.1-fold), indicating that these molecular effects are radiation-dependent. Expression data were normalized to GAPDH for mRNA and U6 for miRNA, as shown in figure 3.

Validation of miR-17-5p targeting of CTNNB1

To confirm the regulatory relationship between miR-17-5p and CTNNB1, dual-luciferase reporter assays were performed. CP-M066 cells were co-transfected with wild-type (WT) or mutant (MUT) CTNNB1 3′UTR constructs and miR-17-5p mimics. In WT constructs, luciferase activity was reduced by 50% ± 0.07 (P<0.05 vs. miR-NC), confirming direct miR-17-5p binding at predicted sites (validated using

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the ENCORI database). No significant reduction was observed in MUT constructs (P>0.05).

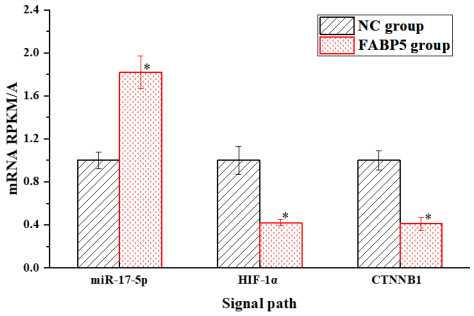

Figure 3. Relative expression (2^-ΔΔCt) of (A) miR-17-5p, (B) HIF1A, and (C) CTNNB1 in various treatment groups compared with control* indicates P < 0.05, ** indicates P < 0.01; bars show mean ± SD.

Furthermore, anti-miR-17-5p transfection increased CTNNB1 expression by 1.8 ± 0.2-fold (P<0.05), confirming negative regulation. Each assay was performed in triplicate, with Renilla luciferase as internal control for normalization. Results are illustrated in figure 4.

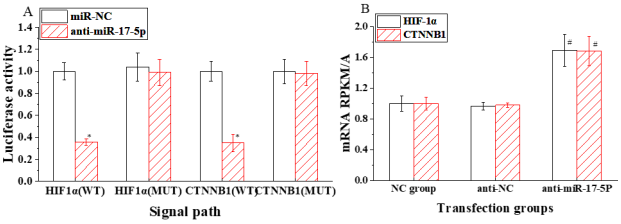

Figure 4. (A) Luciferase activity in WT and MUT CTNNB1 3'UTR constructs with miR-17-5p or NC. **(B)** Relative CTNNB1 mRNA expression after miR-17-5p inhibition. Data are mean ± SD; *P < 0.05 vs. control.

DISCUSSION

Radiation-induced bladder fibrosis (BF) remains a significant late complication in patients undergoing pelvic RT for malignancies [2,21]. Our study reveals that Circ-FABP5 regulates radiation-induced BF through activation of the Wnt/β-catenin signaling pathway via the miR-17-5p/HIF1A/CTNNB1 axis, providing new mechanistic insight into how circRNA-mediated regulation shapes the fibrotic response after irradiation.

Radiation exposure promotes fibroblast activation and ECM deposition, consistent with findings by Fry *et al.* (2018), who described inflammation-driven fibrosis in bladder tissue [1]. The upregulation of α-smooth muscle actin (α-SMA) and collagen I observed here parallels the results of Di *et al.* (2023), who reported collagen accumulation as a hallmark of bladder dysfunction [10]. Circ-FABP5's mitigating effect on these fibrotic markers suggests a potential anti-fibrotic role, consistent with its previously recognized metabolic regulatory functions [5-8, 22, 23].

The Wnt/β-catenin pathway serves as a core driver of fibrosis in multiple organs [9, 24]. Our results show that Circ-FABP5 upregulates miR-17-5p, which subsequently suppresses HIF1A and CTNNB1, inhibiting fibroblast proliferation and ECM deposition. This aligns with the work of Katoh (2018), who emphasized Wnt/β-catenin's central role in organ fibrosis [9, 24]. The direct targeting of CTNNB1 by miR-17-5p, validated by luciferase assays, mirrors observations by Duan *et al.* (2015), who showed miR-133 modulation of TGF-β1 in bladder fibrosis [14, 15]. Unlike these studies, however, ours uniquely integrates radiation as the initiating fibrotic trigger, revealing Circ-FABP5's novel position as a molecular mediator of radiation-modulated Wnt signaling.

HIF1A, an essential regulator of hypoxia and Wnt signaling, promotes fibroblast activation and matrix deposition under stress [11-13, 25]. Li *et al.* (2022) demonstrated that inhibition of HIF1A attenuates neurogenic bladder fibrosis [26], consistent with our finding that Circ-FABP5-mediated suppression of HIF1A alleviates radiation-induced fibrotic responses. Similarly, CTNNB1 (β-catenin) has been widely reported as a fibrosis-promoting effector in multiple pathologies [27]. Yang *et al.* (2019) identified that circ-CTNNB1 enhances β-catenin signaling in cancer cells [27], and our results confirm that silencing CTNNB1 reduces fibroblast proliferation under radiation stress.

Studies by Seo *et al.* (2020) and Zhang *et al.* (2020) revealed that FABP5 is associated with lipid metabolic reprogramming and oncogenic activity, indicating its broader regulatory potential in fibrotic disease [7, 8, 22, 23]. Our data extend these findings to radiation-induced models, suggesting that Circ-FABP5 functions as a master regulator controlling radiation-driven lipid and fibrotic signaling. In contrast, Liu *et al.* (2019) focused on Schwann cell protection in fibrosis [15], further reinforcing that distinct miRNA, including miR-17-5p, play pivotal roles in context-specific bladder pathology.

Radiation's impact on fibrotic injury is well-established. Bentzen (2006) and Jaal & Dörr (2010) described how fractionated pelvic RT induces chronic inflammation and fibrotic tissue remodeling [2, 28]. Our use of 4 Gy radiation, consistent with these models, successfully triggered fibrotic markers without excessive cytotoxicity, bridging preclinical dose–response studies and clinical scenarios [18-20, 29]. Furthermore, Zwaans *et al.* (2016) demonstrated radiation-induced cystitis modeling in mice [30], complementing our in vitro approach.

The therapeutic implications of this study are considerable. Targeting HIF1A and CTNNB1 as downstream nodes of the Circ-FABP5/miR-17-5p axis aligns with Chen *et al.* (2021), who showed that Wnt/β-catenin inhibitors effectively reduce fibrosis in other organs [31]. Moreover, Zhou *et al.* (2020) highlighted the importance of circRNAs in regulating organ fibrosis, supporting Circ-FABP5's novelty as a therapeutic target in radiation-induced settings [32].

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Nonetheless, limitations exist. This study was performed *in-vitro* using a single fibroblast cell line (CP-M066), which cannot fully mimic the cellular heterogeneity and extracellular dynamics of in vivo tissue [33]. The short 48-hour post-irradiation observation period also precludes evaluation of chronic remodeling processes. Future in vivo studies integrating multi-lineage cell interactions and long-term radiation effects will be necessary to validate these mechanisms.

CONCLUSION

Circ-FABP5 regulates radiation-induced bladder fibrosis through the miR-17-5p/HIF1A/CTNNB1 axis and activation of the Wnt/β-catenin signaling pathway. By linking radiation exposure to circRNA-mediated modulation of fibroblast behavior, this study provides a novel molecular framework for understanding radiation-induced bladder dysfunction. These findings suggest that targeting the Circ-FABP5–miR-17-5p–HIF1A/CTNNB1 signaling network could represent a promising therapeutic strategy to mitigate radiation-induced fibrotic damage in elderly patients undergoing pelvic radiotherapy.

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Ethics approval and consent to participate**:** Not applicable. This study involved only in vitro experiments using established mouse fibroblast cell lines and did not include any human participants or animal subjects. Therefore, ethical approval and informed consent were not required.

Consent for publication**:** Not applicable.

Availability of data and materials**:** All data generated or analyzed during this study are included in the main manuscript and supplementary materials. Additional datasets or methodological details are available from the corresponding author upon reasonable request.

Conflict of interest**:** The authors declare that there are no conflicts of interest related to this publication.

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Authors' contributions**:** X.W. and H.W., conceived and designed the study. X.W. and K.P., established the database and experimental design. Data were collected by X.W., J.Y., K.P., and H.W., Data analysis and interpretation were performed by J.Y., H.W., and J.Y., X.W., drafted the initial version of the manuscript, and all authors critically revised the text and approved the final version for publication.

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