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A DNA-PKcs-primary cilia axis maintains ionizing radiation-induced cellular senescence in tumor cells

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Abstract

Senescence is a cellular response closely associated with genotoxic stress that plays a critical role in determining cell fate following irradiation exposure. Primary cilia are sensory organelles on the cell surface capable of detecting and transmitting diverse signaling cues. However, the relationship between primary cilia and senescence in long-term cell fate determination following ionizing radiation (IR) remains unclear. Here, we show that phosphorylated DNA-dependent protein kinase catalytic subunit (p-DNA-PKcs) colocalizes with centromeres at various stages of mitosis, while during interphase, p-DNA-PKcs is restricted to the nucleus of tumor cells. Following irradiation exposure, primary cilia formation is induced and persistently maintained at high levels in senescent tumor cells. Inhibition of DNA-PKcs enhances primary cilia formation, whereas combined siDNA-PKcs inhibition and irradiation reduces cilia generation; furthermore, chloral hydrate-induced primary cilia removal leads to senescent cell death and reduces p-DNA-PKcs protein expression. Notably, treatment with the apoptosis inducer ABT263 also leads to increased cell death and reduced primary cilia incidence. Inhibition of either primary cilia or DNA-PKcs can further enhance the radiosensitivity of tumor cells. These findings suggest that p-DNA-PKcs promotes primary cilia formation post-irradiation and plays a critical role in both the induction and maintenance of cellular senescence.

Full Text

Preamble

A DNA-PKcs-Primary Cilia Axis Maintains Cellular Senescence Induced by Ionizing Radiation in Tumor Cells

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Running title: DNA-PKcs-cilia axis in IR-induced senescence

Abstract

Cellular senescence is a stress response closely associated with genotoxic damage and plays a critical role in determining cell fate following irradiation exposure. Primary cilia, which are sensory organelles on the cell surface, detect and transmit diverse signaling cues. However, the relationship between primary cilia and senescence in long-term cell fate decisions after ionizing radiation (IR) remains poorly understood. Here, we demonstrate that phosphorylated DNA-dependent protein kinase catalytic subunit (p-DNA-PKcs) co-localizes with centromeres during various mitotic stages, while during interphase, p-DNA-PKcs is confined to the nucleus in tumor cells. Following irradiation exposure, primary cilia are formed and persistently maintained at high levels in senescent tumor cells. Inhibition of DNA-PKcs enhances primary cilia formation, while combined inhibition with siDNA-PKcs and irradiation reduces cilia generation. Moreover, chloral hydrate-induced primary cilia removal results in senescent cell death and decreases p-DNA-PKcs protein expression. Notably, treatment with the apoptosis inducer ABT263 also leads to increased cell death and a decreased incidence of primary cilia. Inhibition of either primary cilia or DNA-PKcs further enhances the radiosensitivity of tumor cells. These findings suggest that p-DNA-PKcs contributes to primary cilia formation after irradiation and plays a critical role in both the induction and maintenance of cellular senescence.

Key Words: primary cilia, DNA-PKcs, senescence, ionizing radiation

Introduction

Cellular senescence refers to a stable growth arrest accompanied by an anti-apoptotic state that occurs in response to irreparable stress [1, 2]. Research has demonstrated that ionizing radiation (IR) can induce cellular senescence, which is commonly observed in tumor radiotherapy [3]. Furthermore, cellular senescence triggered by tumor treatments is closely associated with treatment resistance and poor prognosis. Current evidence indicates that activation of the p16–pRB and p53–p21 signaling pathways, mitochondrial dysfunction [4], and cyclic GMP–AMP synthase stimulation are major mechanisms underlying senescence. However, the specific molecular basis of radiation-induced senescence remains incompletely defined.

Previous findings demonstrated that Aurora A acts as a key downstream effector of p21 in radiation-induced senescent tumor cells [5], and its degradation facilitates the formation of primary cilia [6]. These observations suggest that primary cilia may be critical in mediating cellular senescence induced by IR. Primary cilia are hair-like, non-motile organelles that extend from the cell surface [7] and function as central hubs for sensing physical, chemical, and biological cues from the extracellular environment. By transmitting these signals into the cell, primary cilia play a critical role in regulating various cellular processes [8]. Structural or functional defects in primary cilia have been implicated in the development and progression of multiple diseases, including cancer [9].

Numerous studies have demonstrated a strong association between primary cilia dynamics and cellular senescence under physiological conditions. Cilium length increases significantly in aged human fibroblasts and in the kidneys and pancreas of aged mice [10, 11]. A similar elongation has also been observed in the hippocampal region of aged rats [12, 13]. Additionally, silencing ciliogenesis-related genes in primary cilia can induce senescence in renal epithelial cells from mouse models of cystic kidney disease [14]. Notably, further investigations have indicated that primary cilia contribute to the senescence of tumor cells. For instance, persistent primary cilia have been found to induce senescence in human cervical cancer cells [15], and primary cilia play a key role in etoposide-induced senescence in adrenal cortical tumor cells [16].

Although the involvement of primary cilia in cellular senescence has been partially clarified, the precise regulatory mechanisms remain to be fully defined. In normal cells, recent studies have reported a transient increase in cilia formation following irradiation exposure, followed by a gradual decline, and this transient cilia formation plays an essential role in initiating cellular senescence post-irradiation [17]. However, no studies have reported whether primary cilia exhibit similar function or expression patterns during IR-induced tumor cellular senescence.

DNA-PK, ataxia-telangiectasia mutated (ATM), and ATM- and Rad3-related are members of the phosphatidylinositol 3-kinase-related serine/threonine kinase family, which plays a central role in the cellular response to and repair of

DNA damage, particularly double-strand breaks. DNA-PKcs functions as a key component of the non-homologous end-joining repair pathway [18]. In addition to its involvement in DNA damage response and repair, recent studies have demonstrated that DNA-PKcs regulates DNA damage-induced ciliogenesis [19]. Previous findings demonstrated that phosphorylated DNA-PKcs (p-DNA-PKcs) localizes to centrioles during the mitotic phase in glioblastoma cells and dissociates during interphase [20]. Following exposure to IR, p-DNA-PKcs becomes highly expressed and activated, subsequently translocating to the nucleus to mediate DNA repair. Concurrently, the incidence of primary cilia formation in GBM cells significantly increases. These observations support the hypothesis that p-DNA-PKcs binding to centrioles may suppress primary cilia formation, whereas its radiation-induced relocation to the nucleus permits ciliogenesis, indicating a potential regulatory role for p-DNA-PKcs in this process induced by IR.

The study revealed that primary cilia persist stably for extended periods in tumor cells undergoing radiation-induced senescence. Primary cilia arise during the early phase of senescence induction and play a critical role in sustaining the senescent state. The findings further demonstrate that DNA-PKcs is essential for primary cilia formation following irradiation exposure. A deeper understanding of the biological functions of DNA-PKcs-mediated ciliogenesis and its association with radiation-induced cellular senescence may provide important insights for advancing the development and clinical translation of DNA-PKcs-targeted therapies.

Materials and Methods

Cell Culture and Drug Treatments

The human lung cancer cell line A549 was purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). The human GBM-derived GS1910 cells were generated from a GBM patient as previously described [20]. A549 and GS1910 cells were cultured in Dulbecco' s Modified Eagle Medium/F-12 medium (DMEM/F12, Gibco, NY, USA) supplemented with 10% fetal bovine serum (FBS, Beit Haemek, Israel) and maintained at 37 °C in a 5% CO_2 humidified incubator. In all experiments, the growth medium was replaced with fresh medium before irradiation. For long-term cultures, the medium was replaced every 2 days after irradiation. For drug treatments, cells were incubated with 2 M NU7441 (Ku-57788) (DNA-PKcs inhibitor, MCE, New Jersey, USA), 4 mM chloral hydrate (CH; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), or 2 M Navitoclax (ABT-263, MCE).

X-Ray Irradiation

X-ray irradiation was performed using a PXI Precision X-RAD225 system (PXI, North Branford, USA) at a dose rate of 1.98 Gy/min (225 kV, 13.3 mA). The tube was filtered with 2 mm Al and the samples were placed on a disk 50 cm below the filter. All samples were irradiated at room temperature.

RNA Interference
Specific DNA-PK siRNAs and negative control (NC) siRNA were purchased from Ribobio (Guangzhou, China). The sequences were as follows: siDNA-PKcs 1, 5 -GCATCAGGGTTTAATCAGA-3 ; siDNA-PKcs 2, 5 -GTTGGAGCTTACATGCTAA-3 . Specific IFT88 siRNA and a negative control were purchased from Thermo Fisher Scientific (MA, USA). The sequence was as follows: siRNA, 5 -GAAGAAAGCUGUAUUGCCAAUAGUU-3 . The siRNAs were introduced into the cells using Lipofectamine 2000 reagent (Thermo Fisher). siDNA-PKcs and NC siRNA were used at a concentration of 100 nM, while siIFT88 and NC siRNA were used at 50 nM.

Senescence-Associated β-galactosidase (SA-β-Gal) Assay
Senescence was induced by irradiation. Cells were seeded at 2×10^5 cells per dish into 35 mm culture dishes 24 h before irradiation. After 10 Gy X-ray irradiation, the cells were incubated and fixed on days 5, 10, 15, and 20. Senescent cells were identified using a senescence-associated SA-β-Gal kit (Beyotime, Shanghai, China) according to the manufacturer' s protocol. Images were captured using a Leica DMI6000 microscopy system (Wetzlar, Germany). In each experiment, senescent (SA-β-Gal positive) cells were counted in more than 500 cells. All data were obtained from at least three independent experiments.

Western Blot Analysis
Cells were washed twice with pre-cooled PBS, followed by the addition of 200 L RIPA lysis buffer containing protease and phosphatase inhibitors. The lysate was incubated on ice for 5 minutes and then centrifuged in a pre-chilled centrifuge at 12,000 × g for 10 min. The supernatant was heated at 98 °C for 10 min. Lysates were standardized for protein content, separated by SDS-PAGE, and transferred onto PVDF membranes. After blocking with 5% milk, the membranes were probed with primary antibodies overnight at 4 °C and then incubated with secondary antibody. Blots were incubated with the antibodies listed below at a concentration of 1:1,000 (unless otherwise noted) in antibody diluent and visualized using the enhanced chemiluminescence (ECL) procedure. Antibodies used were: p-DNA-PKcs (S2056) (ab18192) from Abcam (Cambridge, UK); IFT88 (13967-1-AP), ARL13B (17711-1-AP), CP110 (12780-1-AP), p21 (10355-1-AP), Aurora A (66751-1-Ig), GAPDH (60004-1-Ig, 1:4000) from Proteintech (Rosemont, USA); γ-tub (T6557) from Sigma-Aldrich; BCL2 (YM3041) from Immunoway (Texas, USA); Cleaved-caspase3 (9661) from Cell Signaling Technology (CST, Boston, USA). Peroxidase-AffiniPure Goat anti-mouse (111-035-144, 1:4000) or anti-rabbit (115-035-146, 1:4000) secondary antibodies were from EpiZyme (Shanghai, China).

Immunofluorescence and Microscopy
2×10^5 cells were plated onto confocal dishes 24 h before irradiation. At designated time points after irradiation, cells were fixed with 4% paraformaldehyde for 10 min and then with pre-chilled methanol at -20 °C for 20 min. The cells were permeabilized with 0.5% Triton X-100 for 10 min and blocked with 5% goat serum for 1 h at room temperature. Primary antibodies were incubated at

room temperature for 1.5 h, followed by secondary antibodies for 1.5 h at room temperature. Cells were stained with 4 ,6-Diamidino-2-Phenylindole (DAPI, Molecular Probes, Eugene, USA). Primary antibodies were used at a concentration of 1:750 (unless otherwise noted) in antibody diluent: p-DNA-PKcs (Abcam, ab18192); γH2AX (Abcam, ab26350; 1:1500); ARL13B (Proteintech, 17711-1-AP); γ-tub (Sigma-Aldrich, T6557). Secondary antibodies were: Goat anti-Rabbit IgG (H+L) conjugated with Alexa Fluor 488 (Proteintech, SA00006-2) or 594 (A11037) from Invitrogen (California, USA), and Goat anti-Mouse IgG (H+L) conjugated with Alexa Fluor 594 (Invitrogen, A11005) or 488 (Invitrogen, 2714439). Fluorescence images were acquired using an ECHO RVL-100-G system (San Diego, USA). In each experiment, more than 50 cilia were measured for length, cilia incidence was calculated in more than 500 cells, and the numbers of γH2AX foci and p-DNA-PKcs foci were counted in at least 50 cells. All data were obtained from at least three independent experiments.

PI Staining

Cells in control and treatment groups were incubated with 10 g/mL PI in petri dishes for 15 min at room temperature in the dark. Dead cells showed red fluorescence, and images were observed and recorded using a DMI6000 (Leica). In each experiment, apoptotic (PI positive) cells were counted in at least 500 cells. All data were obtained from at least three independent experiments.

Cell Growth Curves

A549 or GS1910 cells (1×10^5) were plated into 12-well plates. Adherent cells were cultured and treated with siRNAs, CH, or Nu7441. Cell numbers were counted 0–72 h after irradiation using a Coulter Counter (Beckman, Brea, USA).

Colony Formation Assay

A549 and GS1910 cells in log growth phase were prepared as single-cell suspensions. 2×10^5 cells were plated onto 35 mm dishes. After transfecting siRNAs, kinase inhibitor, or CH combined with 2 Gy irradiation, 200 cells were plated onto 60 mm dishes and cultured continuously for 14 days. Cells were fixed with 4% paraformaldehyde for 10 min and stained with 0.5% crystal violet for 10 min. Colonies were photographed, and those containing >50 cells were counted as survivors. The survival fraction at 2 Gy (SF2) was calculated as: SF2 = colonies counted at 2 Gy / [cells seeded × (PE/100)], where plating efficiency (PE) of Control (Ctrl) = colony number/plated cell number × 100.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.0.2. All results are reported as mean ± standard deviation (SD). Statistical significance was determined by Student' s t-test or one-way ANOVA, and *$P < 0.05$ was considered statistically significant. All experiments were performed in at least three independent biological replicates.

Results

Senescent Tumor Cells Induced by IR Preserve Primary Cilia

Recent findings demonstrated increased ciliogenesis during senescence induction by IR in A549 and GS1910 cells [20]. To investigate the status of primary cilia during senescence maintenance, A549 and GS1910 cells were exposed to 10 Gy of X-rays, followed by SA-β-Gal staining on days 5, 10, 15, and 20 post-irradiation. The results showed that the proportion of senescent (SA-β-Gal positive) cells remained above 80% in both cell lines at 5 days after irradiation (Figure 1A [Figure 1: see original paper]-C). Consistently, the protein expression level of p21, one of the main proteins involved in senescence [21], was continually upregulated, while expression of Aurora A, a downstream target of p21 involved in ciliogenesis suppression [5], was markedly suppressed (Figure 1D), indicating a stable senescent status in irradiated cells.

Notably, persistent and robust ciliogenesis was detected in senescent tumor cells (Figure 1E). The incidence of primary cilia reached approximately 70% in senescent A549 and GS1910 cells (Figure 1F and 1H). Additionally, the average cilium length increased from 1.5 ± 0.9 m to 3.0 ± 1.1 m in A549 cells and from 1.5 ± 0.9 m to 2.8 ± 1.3 m in GS1910 cells (Figure 1G and 1I). In senescent cells, elevated expression levels of IFT88 and ARL13B, two key regulators of primary cilia assembly and function [22, 23], were also observed (Figure 1J). These findings suggest that sustained ciliogenesis induced by IR is associated with the initiation of senescence and its long-term maintenance.

Relationship Between Ciliogenesis and DNA-PKcs

DNA-PKcs is involved in mitosis [24]. Previous studies have reported that DNA damage-induced primary cilia formation depends on both the activation and centrosomal localization of p-DNA-PKcs [19]. Earlier results suggested that the activation status and spatial distribution of p-DNA-PKcs may be involved in primary cilia formation following irradiation exposure [20]. To further investigate the correlation between p-DNA-PKcs and the initiation of ciliogenesis, the subcellular localization of p-DNA-PKcs was examined. p-DNA-PKcs was observed to localize to the centrosome at the beginning of the M phase and possibly during the interdivision phase until the end of the M phase, following the centrosome to be evenly distributed to daughter cells in GS1910 cells (Figure 2A [Figure 2: see original paper]). In contrast, during non-mitotic phases, p-DNA-PKcs was predominantly detected within the nucleus (Figure 2B [Figure 2: see original paper]). These findings suggest that p-DNA-PKcs may contribute to disassembling primary cilia or suppressing their formation.

Furthermore, upon IR-induced DNA damage, p-DNA-PKcs was activated and translocated from the centriole to the nucleus (Figure 2C [Figure 2: see original paper]), supporting the hypothesis that p-DNA-PKcs may act as a negative regulator of ciliogenesis.

Impact on Primary Ciliogenesis After DNA-PKcs Interference

To investigate whether p-DNA-PKcs inhibits the formation of primary cilia,

DNA-PKcs expression was suppressed by transfecting cells with siRNA specifically targeting it. A reduction in DNA-PKcs levels was confirmed by western blotting and immunofluorescence staining (Figure 3A [Figure 3: see original paper]-E). In cells treated with siDNA-PKcs combined with irradiation, an increased number of γH2AX foci was observed, accompanied by reduced nuclear translocation of p-DNA-PKcs (Figure 3D-E), indicating diminished damage repair capacity following DNA-PKcs interference.

Subsequently, primary cilia incidence and average length were assessed in A549 and GS1910 cells after siDNA-PKcs treatment (Figure 3F). The incidence of primary cilia reached approximately 45% in A549 cells and approximately 30% in GS1910 cells (Figure 3G and 3I). Additionally, the average cilia length increased from 1.3 ± 0.7 m to 2.6 ± 1.1 m in A549 cells and from 1.1 ± 0.7 m to 1.5 ± 0.7 m in GS1910 cells, respectively (Figure 3H and 3J). To further validate these observations, expression levels of IFT88 and ARL13B were examined and found to be upregulated, while CP110, a negative regulator of ciliogenesis [25], was downregulated in both cell lines following siDNA-PKcs treatment (Figure 3K). These findings suggest that DNA-PKcs inhibits the formation of primary cilia.

Furthermore, changes in primary cilia were evaluated following combined treatment with irradiation and siDNA-PKcs. A reduction in the incidence and length of primary cilia was observed in A549 and GS1910 cells under combined treatment conditions (Figure 3G-J). Since DNA-PKcs participates in DNA damage repair following irradiation, the formation of γH2AX foci at double-strand break sites was monitored over time (Figure 3L). An increased number of γH2AX foci was detected at multiple time points after combined treatment, indicating enhanced irradiation sensitivity (Figure 3M-N). These results suggest a decline in DNA repair efficiency and an increased likelihood of cell death. To further explore the survival status of tumor cells, Propidium iodide (PI) staining revealed a marked increase in cell death following siDNA-PKcs combined with irradiation (Figure 3O). The proportion of apoptotic cells increased to 15.4 ± 2.8% and 12.8 ± 1.6% in A549 cells and to 13.5 ± 1.5% and 10.5 ± 0.6% in GS1910 cells (Figure 3P).

Collectively, the data indicate that although the dissociation of DNA-PKcs from the centriole may permit primary cilia formation, functional inhibition of DNA-PKcs impairs DNA repair and enhances cell death, ultimately leading to a decrease in ciliogenesis.

Removal of Primary Cilia Induces Cell Death

Primary cilia remained elevated and sustained over time following irradiation, and p-DNA-PKcs was involved in DNA damage-induced ciliogenesis. To investigate whether p-DNA-PKcs contributes to the maintenance of cellular senescence, chloral hydrate (CH), a small molecule commonly used to remove primary cilia [26], was introduced to ablate primary ciliogenesis (Figure 4A [Figure 4: see original paper]-B). Before CH treatment, apoptosis was rarely detected, while the proportion of apoptotic cells increased to 18.9 ± 0.1% in A549 cells and

12.5 ± 0.3% in GS1910 cells following CH treatment (Figure 4C-D). Analysis of apoptosis-related proteins revealed a decrease in BCL2 expression and an increase in Cleaved-caspase3 following CH treatment (Figure 4E), confirming the induction of apoptosis. Notably, the protein expression level of p-DNA-PKcs was elevated after irradiation but significantly reduced upon primary cilia removal by CH (Figure 4E). These results indicate that the emergence of primary cilia under genotoxic stress conditions may activate DNA-PKcs, suggesting a potential interdependent regulatory relationship between primary cilia and DNA-PKcs.

To further assess the relationship between apoptosis and primary cilia, cell death was induced using the apoptosis-inducing agent ABT263. Following treatment, primary cilia incidence in A549 and GS1910 cells decreased (Figure 4F-G), accompanied by downregulation of primary cilia-related proteins IFT88 and ARL13B in both cell lines (Figure 4J). Furthermore, PI staining indicated an increase in apoptosis after ABT263 treatment, with the proportion of apoptotic cells rising to 33.6 ± 0.8% in A549 cells and 34.6 ± 0.4% in GS1910 cells (Figure 4H-I).

Interference with Primary Cilia or DNA-PKcs Enhances the Radiosensitivity of Cells

After DNA damage caused by irradiation, DNA-PKcs is fully activated and enters the nucleus to respond to and repair DNA, maintaining cell survival. To assess the effect of interfering with primary cilia or DNA-PKcs on radiosensitivity, primary cilia were disrupted using CH or si-IFT88. In both cases, the surviving fraction at 2 Gy (SF2) was significantly reduced in tumor cells (Figure 5A [Figure 5: see original paper] and 5C). Similar results were observed upon DNA-PKcs inhibition using either siRNA or the pharmacological inhibitor NU7441, leading to decreased SF2 levels in A549 and GS1910 cells (Figure 5B and 5D). Further analysis of cell proliferation revealed that interference with either primary cilia or DNA-PKcs significantly reduced the proliferative capacity of tumor cells (Figure 5E-F). These findings suggest that inhibition of DNA-PKcs impairs DNA repair capacity under irradiation stress, thereby reducing both cell proliferation ability and clonogenic survival. Collectively, the data indicate that targeting primary cilia or DNA-PKcs can enhance cellular radiosensitivity.

Discussion

The findings of this study demonstrate that primary cilia are sustained at high levels for prolonged periods in tumor cells undergoing IR-induced senescence. Under physiological circumstances, DNA-PKcs suppresses the formation of primary cilia. In response to IR-induced genotoxic stress, activated DNA-PKcs (p-DNA-PKcs) translocates to the nucleus, promoting primary cilia formation and supporting the maintenance of cellular senescence. Moreover, sustaining primary cilia formation and senescence following irradiation are essential for preserving the DNA damage response. These results underscore the role of DNA-PKcs-mediated long-term ciliogenesis in maintaining cellular senescence

during IR-induced injury.

After irradiation, the primary cilia of senescent tumor cells increased and persisted for an extended period. Disruption of these primary cilia resulted in increased cell death, suggesting that primary cilia may contribute to the maintenance of the senescent state. Primary cilia are rarely observed under normal conditions in human fibroblasts but become more prominent during senescence [10]. In contrast, primary cilia are absent in age-related condylar cartilage degeneration [27]. These observations suggest that cells with abundant primary cilia under physiological conditions may depend on primary cilia-related signaling pathways to support proliferation. Primary cilia loss in such cells may impair proliferative capacity, thereby triggering entry into senescence. Conversely, in cells that do not require primary cilia for normal proliferation, an increase in primary cilia may interfere with cell cycle progression and induce senescence through cell cycle arrest.

Previous studies have reported that p-DNA-PKcs located at the primary cilia base can promote cilia formation and are essential for its initiation, with p-DNA-PKcs found in both the cytoplasm and nucleus [19]. In contrast, the present study revealed that p-DNA-PKcs localized to centrioles promotes mitosis progression. Notably, p-DNA-PKcs was observed on centrioles during the G2 phase, a stage at which primary cilia begin to depolymerize. Other studies have suggested that DNA-PKcs facilitates entry into and exit from mitosis by stabilizing centrosomal structure and supporting mitotic processes [28, 29]. Therefore, in the absence of external stress signals, centriole-associated DNA-PKcs may promote cilia depolymerization. Experimental results further indicated that p-DNA-PKcs was exclusively localized on centrioles during the M phase and restricted to the nucleus during all other cell cycle phases. Upon IR-induced DNA damage, p-DNA-PKcs translocated into the nucleus to participate in DNA damage repair and support cell survival. These findings support the possibility that DNA-PKcs contributes to the initiation of ciliogenesis under stress conditions, while in normal conditions, DNA-PKcs may facilitate mitosis by promoting primary cilia disassembly or suppressing its formation. Further investigation is required to validate this hypothesis.

Following irradiation exposure, a significant increase in primary cilia formation was observed. However, when DNA-PKcs inhibition was combined with irradiation, the incidence of primary cilia was reduced rather than enhanced. These results suggest that although the dissociation of DNA-PKcs from centrioles provides a foundation for ciliogenesis, DNA-PKcs inhibition compromises DNA repair capacity, leading to increased cell death and consequently a reduction in ciliogenesis. Moreover, removal of cilia using CH led to a decrease in p-DNA-PKcs expression, suggesting that ciliogenesis under conditions of cellular damage may activate DNA-PKcs. These observations point to a potential bidirectional regulatory effect between primary cilia and DNA-PKcs.

The occurrence of primary cilia is related to the cell cycle, particularly during the quiescent phase [30]. Consequently, most cancers lack primary cilia [31].

However, several studies have identified primary cilia expression in specific tumor types [32-34], with functional roles varying depending on the tumor type. Previous research has demonstrated that primary cilia are closely involved in tumor initiation and progression [17], resistance to molecularly targeted therapies [35], and resistance to radiotherapy and chemotherapy [6, 36]. IR-induced cellular senescence is widely observed during tumor treatment, and experimental findings in the present study confirmed the induction of senescence in tumor cells following irradiation exposure.

Early studies suggested that cellular senescence is beneficial for inhibiting tumor development [37]. However, some research has shown that senescent cells may also contribute to carcinogenesis and pathological proliferation [38-40]. In addition, senescent tumor cells have been associated with therapy resistance [41]. Therefore, inducing apoptosis in senescent tumor cells represents an alternative strategy for addressing irradiation resistance in tumors. Previous studies have demonstrated that primary cilia regulate both cellular senescence and apoptosis [15, 42], and some investigations have indicated that IR-induced primary cilia formation is associated with the initiation of cellular senescence [17]. In the present study, inhibiting primary cilia resulted in the death of senescent tumor cells, suggesting that primary cilia are essential for maintaining the senescent phenotype following irradiation exposure. Moreover, interference with either primary cilia or DNA-PKcs enhanced radiosensitivity, indicating that DNA-PKcs-mediated ciliogenesis contributes to the induction and maintenance of senescence and the development of radioresistance. However, the interplay between primary cilia, tumor cell radioresistance, and the mechanism underlying IR-induced cellular senescence remains poorly understood. Consequently, systematic research is warranted to elucidate the mechanisms by which primary cilia induce cellular senescence post-irradiation and to uncover their role in tumor cell radioresistance. Such insights would provide valuable theoretical foundations and experimental evidence for advancing clinical strategies in cancer treatment.

Based on these insights, we propose that under normal conditions, p-DNA-PKcs localized to centrioles facilitates mitosis, leading to inhibition of ciliogenesis. IR-induced DNA damage dissociates p-DNA-PKcs from the centriole and recruits p-DNA-PKcs into the nucleus, resulting in ciliogenesis and DNA damage repair. Consequently, cellular resistance to IR is enhanced, probably associated with senescence induction (Figure 6 [Figure 6: see original paper]).

In summary, the findings reveal that primary cilia are stably maintained for extended periods following irradiation exposure, while nuclear translocation of p-DNA-PKcs sustains the DNA damage response and facilitates primary cilia formation. This dual role is essential for preserving cellular senescence and promoting tumor cell survival. The study elucidates the mechanism of IR-induced primary cilia formation and identifies a novel function of DNA-PKcs in regulating ciliogenesis and sustaining the senescence response.

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Conflict of Interests

The authors declare no conflicts of interest regarding this manuscript.

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Figure Legends

Figure 1. Senescent tumor cells induced by IR preserved primary cilia. (A) SA-β-Gal staining of A549 and GS1910 cells treated with 10 Gy of X-rays. Scale bar, 10 m. (B-C) Quantification of SA-β-Gal positive staining in A549 and GS1910 cells. (D) Western blot analysis of p21 and Aurora A protein expression in A549 and GS1910 cells. (E-I) Immunofluorescence staining and quantitative analysis of primary cilia in A549 and GS1910 cells. Scale bar, 10 m. (J) Western blot analysis of IFT88 and ARL13B protein expression in A549 and GS1910 cells. All data were obtained from at least three independent experiments and shown as mean $\pm$ SD. ***P $<$ 0.001.

Figure 2. DNA-PKcs and primary ciliogenesis in A549 and GS1910 cells. (A) Immunofluorescence staining for the localization of p-DNA-PKcs during the mitotic phase in GS1910 cells. Scale bar, 5 m. (B) Immunofluorescence staining for the localization of p-DNA-PKcs in GS1910 cells during the interphase period. Scale bar, 5 m. (C) Immunofluorescence staining for the localization of p-DNA-PKcs in A549 and GS1910 cells after treatment with 10 Gy of X-rays. Scale bar, 10 m.

Figure 3. Effects of DNA-PKcs on the generation of primary cilia. (A) Western blot analysis of DNA-PKcs expression following siDNA-PKcs transfection in A549 and GS1910 cells. (B-C) Western blot and quantitative analysis of p-DNA-PKcs expression following combined treatment with siDNA-PKcs and irradiation in A549 and GS1910 cells. (D-E) Immunofluorescence staining and quantitative analysis of the co-localization of p-DNA-PKcs and γH2AX in A549 and GS1910 cells after siDNA-PKcs combined with irradiation. Scale bar, 10 m. (F-J) Immunofluorescence staining and quantitative analysis of primary cilia in A549 and GS1910 cells after siDNA-PK 1, siDNA-PK 2, with or without irradiation. Scale bar, 10 m. (K) Western blot analysis of IFT88, ARL13B, and CP110 protein expression in A549 and GS1910 cells following siDNA-PKcs treatment. (L-N) Immunofluorescence staining of γH2AX foci in DSB regions formed over time after 10 Gy X-ray irradiation in A549 and GS1910 cells. Scale bar, 10 m. (O-P) PI staining and quantitative analysis of dead A549 and GS1910 cells after siDNA-PKcs combined with irradiation. Scale bar, 60 m. All data were obtained from at least three independent experiments and shown as mean ± SD. *$P < 0.05$,* ***$P < 0.01$,*** $P < 0.001$.

Figure 4. Primary cilia deficiency induced cell death. (A-B) Immunofluorescence staining and quantitative analysis of primary cilia in A549 and GS1910 cells after irradiation combined with CH intervention. Scale bar, 10 m. (C-D) PI staining and quantitative analysis of dead A549 and GS1910 cells after irradiation combined with CH intervention. Scale bar, 100 m. (E) Western blot analysis of p-DNA-PKcs, BCL2, and Cleaved-caspase3 protein expression in A549 and GS1910 cells. (F-G) Immunofluorescence staining and quantitative analysis of primary cilia in A549 and GS1910 cells after irradiation combined with ABT263 intervention. Scale bar, 10 m. (H-I) PI staining and quantitative analysis of dead A549 and GS1910 cells after irradiation combined with ABT263 intervention. Scale bar, 100 m. (J) Western blot analysis of IFT88 and ARL13B protein expression in A549 and GS1910 cells. All data were obtained from at least three independent experiments and shown as mean ± SD. ***$P < 0.001$.

Figure 5. Disruption of primary cilia formation or DNA-PKcs increases the radiosensitivity of tumor cells. (A-D) Colony formation assay and SF2 value analysis in A549 and GS1910 cells after 2 Gy X-ray treatment and treatment with IFT88 siRNA/CH or siDNA-PKcs/NU7441. (E-F) Cell proliferation curves of A549 and GS1910 cells. All data were obtained from at least three independent experiments and shown as mean ± SD. *$P < 0.05$,* ***$P < 0.01$,*** $P < 0.001$.

Figure 6. p-DNA-PKcs induces tumor radioresistance by promoting primary cilia formation. Under physiological conditions, p-DNA-PKcs binds to centrioles to facilitate mitosis, leading to inhibition of ciliogenesis. IR-induced DNA damage dissociates p-DNA-PKcs from the centriole and recruits p-DNA-PKcs into the nucleus, resulting in ciliogenesis and DNA damage repair. Consequently, cellular resistance to IR is enhanced, probably associated with senescence induction.

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