Circ-ABCC4 contributes to prostate cancer progression and radioresistance by mediating miR-1253/*SOX4* cascade

Tao Yu[a], Hong Du[a] and Changhai Sun[b]

Circular RNAs (circRNAs) exert pivotal functions in many malignancies. However, the roles of circ-ABCC4 in prostate cancer (PCa) radioresistance and progression remain largely unclear. Cell viability, proliferation, apoptosis, invasion, and radioresistance were evaluated by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, 5-ethynyl-2'-deoxyuridine, flow cytometry, transwell invasion, and colony formation assays. Tumor xenograft experiment was conducted to assess circ-ABCC4 role in xenograft growth *in vivo*. Dual-luciferase reporter assay was implemented to test the target relation of microRNA-1253 (miR-1253) and circ-ABCC4 or SRY-box transcription factor 4 (*SOX4*). Circ-ABCC4 enrichment was prominently raised in PCa tissue specimens and cells. Circ-ABCC4 depletion blocked PCa cell viability, proliferation, invasion, and radioresistance and triggered apoptosis. Circ-ABCC4 silencing aggravated irradiation-induced inhibitory effect on xenografts growth. miR-1253 was a downstream molecule of circ-ABCC4, and circ-ABCC4 depletion-mediated anti-cancer impacts in PCa cells were partly counteracted by decreasing miR-1253 abundance. miR-1253 targeted *SOX4* mRNA, and miR-1253 blocked PCa cell malignant phenotypes partly by targeting SOX4. Circ-ABCC4 could enhance SOX4 abundance by absorbing miR-1253. Circ-ABCC4 exerted a pro-tumor activity by facilitating PCa cell viability, proliferation, invasion, and radioresistance and suppressing apoptosis. *Anti-Cancer Drugs* 34: 155–165

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Introduction

Currently, there is no standard method for the early diagnosis and therapy of prostate cancer (PCa) patients [1]. The prognosis of PCa cases remains undesirable [2,3]. Metastasis and radioresistance cause the recurrence of PCa. Therefore, elucidating the mechanism behind PCa progression is essential to improve patients' outcomes.

Circular RNAs (circRNAs) are firstly regarded as the by-products of gene transcription [4]. Currently, an increasing number of circRNAs were identified and they were found to take part in many cellular processes [5–7]. CircRNAs are dysregulated in malignancies, and circRNAs can play vital roles in carcinogenesis and progression [8]. For instance, circ_0001206 expression declined in PCa, and circ_0001206 restrained PCa cell growth and metastasis [9]. Huang *et al*. found that circ-ABCC4 aggravated PCa development by modulating microRNA-1182 (miR-1182)/*FOXP4* axis [10]. Here, the mechanism of circ-ABCC4 in PCa development was further tested.

MicroRNAs (miRNAs) have been found to regulate the progression of PCa by previous works. For example, miR-182 accelerated PCa development via activating Wnt/β-catenin pathway [11]. miR-375 enhanced the chemoresistance of PCa cells to docetaxel through modulating *SEC23A* and *YAP1* [12]. MicroRNA-1253 (miR-1253) was reported to act as the target of FOXC2-AS1 to inhibit the development of PCa cells via *EZH2* [13]. However, miR-1253 function in PCa is still largely unclarified.

SRY-box transcription factor 4 (SOX4) was initially shown to regulate embryonic development and cell fate decisions [14,15]. The abnormal upregulation of *SOX4* was observed in many malignancies, containing PCa [16]. Feng *et al*. indicated that miR-19a-3p restrained PCa cell metastasis by targeting and suppressing *SOX4* [17]. miR-130a was found to elevate the radiosensitivity in rectal cancer cells via *SOX4* [18]. Here, the target relation of miR-1253 and *SOX4* was testified, and the mechanism of SOX4 in PCa was explored.

We intended to probe into the role and potential mechanism of circ-ABCC4 in PCa. Circ-ABCC4 enrichment was notably enhanced in PCa. Knockdown assays revealed that circ-ABCC4 depletion blocked PCa cell viability, proliferation, invasion, and radioresistance and

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triggered apoptosis. Subsequently, the miRNA/mRNA cascade downstream of circ-ABCC4 was foretold by the bioinformatics tool and was verified through compensation assays.

Materials and methods

Patient specimens

PCa tissue specimens (60 cases) and paired para-cancer non-tumor specimens (60 cases) were acquired from 60 PCa cases at Weihai Central Hospital. The procedures were authorized by the Medical Ethics Committee of Weihai Central Hospital. All the cases had signed the written informed consent.

Cell lines

DU145, PC3, VCaP, and 22Rv1 along with RWPE-1 were acquired from BeNa Culture Collection (Beijing, China) and were then maintained in dulbecco's modified eagle medium (Gibco, Carlsbad, California, USA) added with 10% fetal bovine serum (FBS) (Gibco).

RT-qPCR

Prime Script RT Master Mix (Takara, Dalian, China) and ReverAid First Strand cDNA Synthesis (Thermo Fisher Scientific, Mountain View, California, USA) were used to synthesize cDNA which was used as the template of PCR. SYBR Green Mix (Thermo Fisher Scientific) was utilized to quantify the abundance of circ-ABCC4, miR-1253, and *SOX4* mRNA. The abundance was analyzed by $2^{-\Delta\Delta Ct}$. All primers are shown in Table 1.

Small RNAs and plasmids

Circ-ABCC4-specific shRNA (sh-circ-ABCC4) and sh-NC, circ-ABCC4 re-constructed plasmid (circ-ABCC4) and pLCDH-cir, miR-1253 and miR-NC, anti-miR-1253 and anti-miR-NC, *SOX4* re-constructed expressing vector (SOX4) and pcDNA were acquired from Genechem Company (Shanghai, China) and Genepharma (Shanghai, China). The day after seeding PCa cells, Lipofectamine 3000 (Invitrogen, Carlsbad, California, USA) was adopted for transfection.

MTT assay

After transfection for 48 h, 20 µL 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) (Life Technologies, Waltham, Massachusetts, USA) was pipetted, and PCa cells were mixed with MTT agent for 4 h. Cell supernatant was removed, and 150 µL dimethyl sulfoxide (Sangon Biotech, Shanghai, China) was pipetted to wells. The optical density was examined at 570 nm. This experiment was implemented in triplicates.

EdU assay

PCa cells were mixed with 5-ethynyl-2'-deoxyuridine (EdU) reagent (keyGEN Biotech, Jiangsu, China) for 2 h. Subsequently, the nucleus was dyed via 4',6-diamidino-2-phenylindole (Sigma, St. Louis, Missouri, USA). The fluorescence intensities were observed on a fluorescence microscope (Leica, Wetzlar, Germany).

Flow cytometry

Annexin V-FITC Apoptosis Kit (BD Biosciences, San Jose, California, USA) was adopted. Annexin V-FITC (5 µL) and PI (5 µL) were pipetted to mark phosphatidylserine and DNA content. Cell apoptosis was evaluated by FACSCalibur (Becton Dickinson, Franklin Lakes, New Jersey, USA).

Transwell invasion assay

Transwell assay was implemented with commercial Matrigel-coated transwell compartments (BD Biosciences). PCa cells (2×10^4 cells) were dispersed in 100 µL serum-free media. Cell suspension was pipetted to the above compartments. The below compartments were padding with 600 µL media plus 10% FBS (Gibco). After 24 h, invaded cells at 100× were counted.

Colony formation assay

PCa cells in 6 cm plates were exposed to increased doses of irradiation via 6-MV therapeutic linear accelerator (Varian, San Jose, California, USA). Equal amounts of PCa cells were plated onto six-well plates (1×10^4 cells/well) to incubate for 14 days. These colonies were dyed with crystal violet (Sangon Biotech).

Tumor xenograft experiment

Twenty-eight BALB/c nude mice were acquired from Vital River Laboratory Animal Technology (Beijing, China). PC3 cells with the stable insertion of sh-circ-ABCC4 or sh-NC were built. A total of 3×10^6 PC3 cells were inoculated to the nude mice. After 7 days, 5 Gy of irradiation was delivered to the nude mice every 4 days. Meanwhile, tumor dimension was examined by caliper measurement and analyzed as $\text{volume} = \text{length} \times \text{width}^2 \times 0.5$. After 27 days, xenografts were dissected for expression

Table 1 Primers in quantitative real-time polymerase chain reaction

Gene	Species	Forward primer (5′–3′)	Reverse primer (5′–3′)
circ-ABCC4	Human	TCAATTCTGAAAGCTCCGGTA	CAGTGATGACTTCCCTGCTC
miR-1253	Human	GGGCAGAGAAGAAGATCA	GCAGGGTCCGAGGTATTC
SOX4	Human	GCACTAGGACGTCTGCCTTT	ACACGGCATATTGCACAGGA
U6	Human	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAT
GAPDH	Human	CCTGTTCGACAGTCAGCCG	GAGAACAGTGAGCGCCTAGT

GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

detection of circ-ABCC4 via RT-qPCR. The protocols were authorized by the Institutional Animal Care and Use Committee of Weihai Central Hospital.

Dual-luciferase reporter assay

The fragment of circ-ABCC4 or the 3′ untranslated region (3′UTR) of *SOX4* which contains the supposed miR-1253-binding sequence was synthesized and constructed into psiCHECK2 vector (Promega, Madison, Wisconsin, USA), named as WT-circ-ABCC4 or SOX4 3′UTR-WT. MUT-circ-ABCC4 and SOX4 3′UTR-MUT were constructed in which miR-1253-binding sequence was mutated. A total of 10 nM small RNAs were introduced with 40 ng reporter plasmids into PCa cells. Luciferase activities were measured via a commercial kit (Promega).

Western blot assay

Cell lysis buffer (Abcam, Cambridge, Massachusetts, USA) was adopted to prepare protein samples. Protein supernatant was isolated from cell debris via centrifugation at 13 200*g* for 15 min. A BCA Kit (Pierce, Rockford, Illinois, USA) was adopted to quantify protein samples. Proteins were added to SDS-PAGE gel and shifted onto polyvinylidene fluoride membrane (Millipore, Billerica, Massachusetts, USA). Primary antibodies, including anti-CyclinD1 (ab16663; Abcam), anti-SOX4 (ab243041; Abcam), and anti-glyceraldehyde 3-phosphate dehydrogenase (ab8245; Abcam), were diluted in 3% BSA and then mixed with the membrane overnight at 4°C. The membrane was labeled with the secondary antibody for 2 h. Immunoreactive protein bands were determined by the ECL Kit.

Data analysis

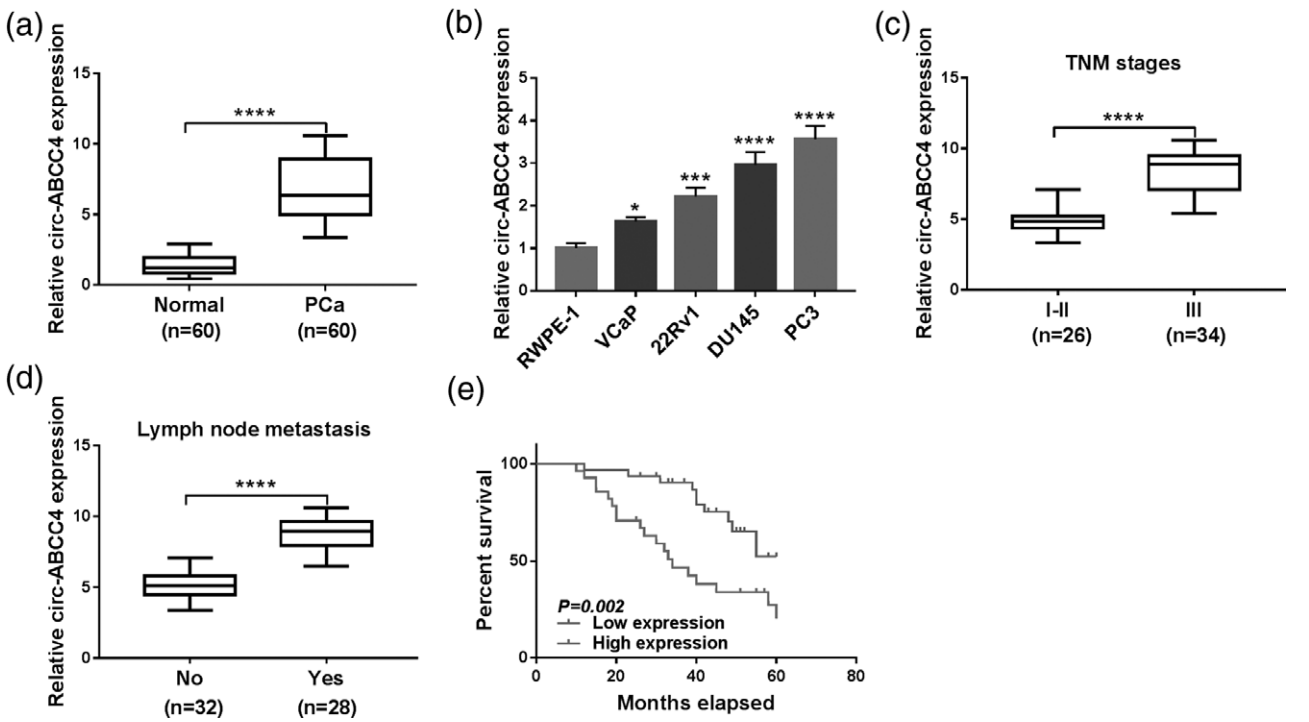
Statistical data were exhibited as mean ± SD. Difference was evaluated with paired and unpaired Student's *t*-test or one-way analysis of variance. Differences were identified as significant at $P < 0.05$.

Results

High abundance of circ-ABCC4 may be an indicator of poor prognosis in prostate cancer patients

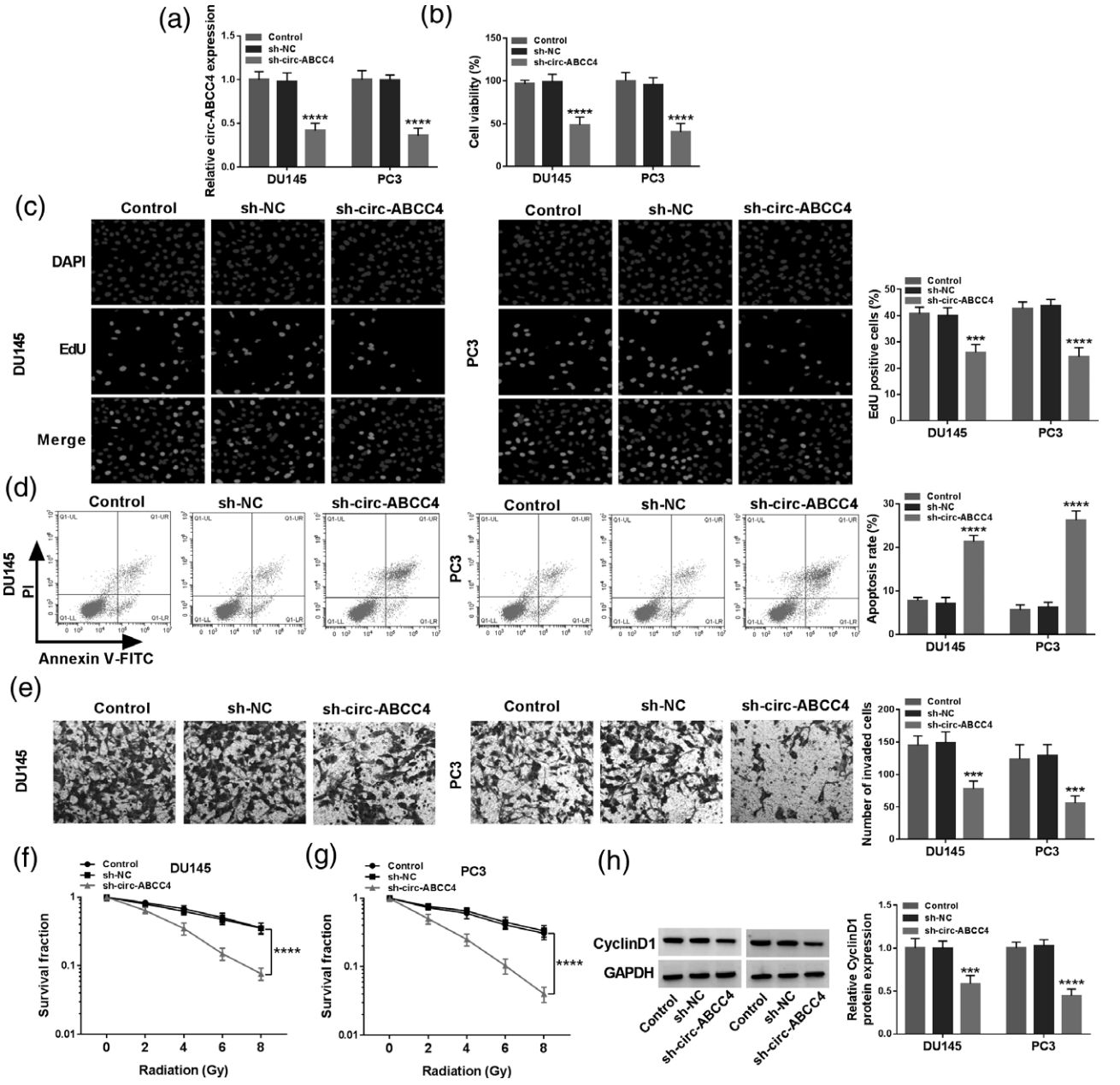
Circ-ABCC4 expression was enhanced in PCa tissue specimens ($n = 60$) (Fig. 1a). Also, circ-ABCC4 abundance was enhanced in four PCa cell lines (Fig. 1b). Circ-ABCC4 expression was notably enhanced in PCa

Fig. 1



High abundance of circ-ABCC4 may be an indicator of poor prognosis in PCa cases. (a) Circ-ABCC4 abundance was examined in 60 pairs of PCa tissue specimens and adjacent tissue specimens via RT-qPCR. (b) Circ-ABCC4 abundance in PCa and RWPE-1 cells was examined via RT-qPCR. (c) PCa cases were divided into two groups based on TNM stages, and circ-ABCC4 level was presented. (d) Circ-ABCC4 abundance in PCa cases with positive lymph node metastasis or not was shown. (e) Percent survival of PCa cases with high or low abundance of circ-ABCC4 in five years was shown. *$P<0.05$, ***$P<0.001$, ****$P<0.0001$.

Fig. 2



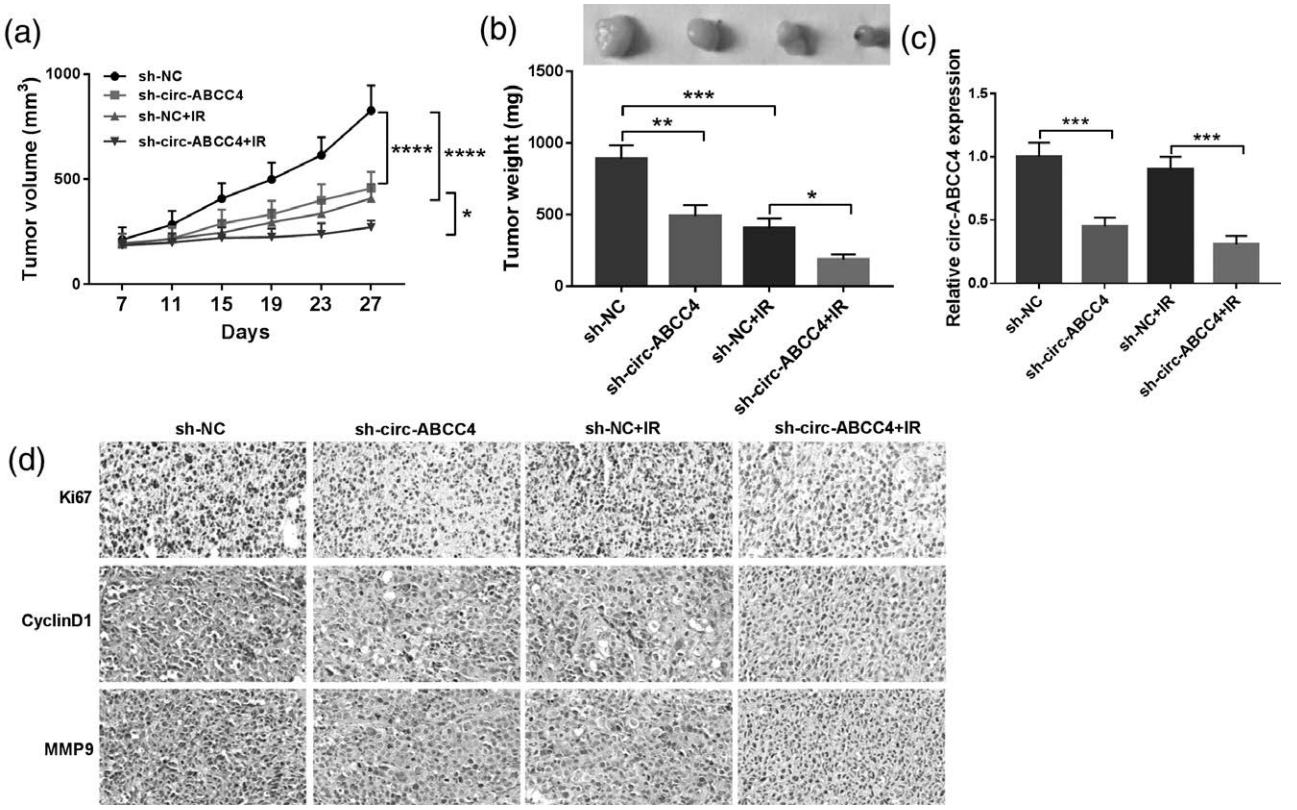
Circ-ABCC4 silencing hampers PCa cell viability, proliferation, invasion, and radioresistance while inducing apoptosis. (a–h) PCa cells were stably introduced with sh-NC or sh-circ-ABCC4. (a) Circ-ABCC4 enrichment in PCa cells was examined by RT-qPCR. (b) Cell viability was measured via MTT assay. (c) Cell proliferation was evaluated via EdU assay. (d) Flow cytometry was implemented to analyze PCa cell apoptosis rate. (e) Transwell assay was implemented to analyze cell invasion. (f and g) Colony formation assay was adopted to evaluate PCa cell radioresistance. (h) The abundance of CyclinD1 protein was determined by western blot. ***$P<0.001$, ****$P<0.0001$. EdU, 5-ethynyl-2'-deoxyuridine; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide.

patients in advanced stage (III stage) and PCa patients with lymph node metastasis (Fig. 1c and d). We analyzed the survival curve of PCa cases with high or low abundance of circ-ABCC4. PCa patients with high circ-ABCC4 abundance were correlated with low survival rate (Fig. 1e). Overall, circ-ABCC4 might be a novel prognostic factor in PCa.

Circ-ABCC4 silencing hampers prostate cancer cell viability, proliferation, invasion, and radioresistance whereas induces apoptosis

Knockdown assays were conducted with sh-circ-ABCC4 to explore its biological role. The interference efficiency of sh-circ-ABCC4 was validated via RT-qPCR (Fig. 2a). After silencing circ-ABCC4, cell viability was markedly

Fig. 3



Circ-ABCC4 interference contributes to irradiation-induced suppressive effect in xenografts growth *in vivo*. (a) Tumor dimension was recorded. (b) Xenografts were weighed after dissecting the mice after a 27-day injection. (c) Circ-ABCC4 expression in four groups was examined via RT-qPCR. (d) IHC assay was carried out to evaluate protein abundance in xenograft tissue specimens. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$.

reduced (Fig. 2b). EdU assay showed that circ-ABCC4 depletion decreased EdU positive cell ratio (Fig. 2c), indicating that circ-ABCC4 knockdown restrained PCa cell proliferation. The apoptosis rate in circ-ABCC4-silenced cells was conspicuously elevated (Fig. 2d). As displayed in Fig. 2e, invaded cell number was reduced by silencing circ-ABCC4, proving that circ-ABCC4 knockdown suppressed PCa cell invasion. PCa cells were irradiated with increased doses, and these cells were subjected to colony formation assay to explore circ-ABCC4 function on PCa cell radioresistance. The survival fraction dramatically declined in circ-ABCC4 silencing group (Fig. 2f and g). Circ-ABCC4 depletion reduced CyclinD1 abundance (Fig. 2h), further proving that circ-ABCC4 interference restrained PCa cell growth. Overall, circ-ABCC4 absence restrained PCa cell malignant properties.

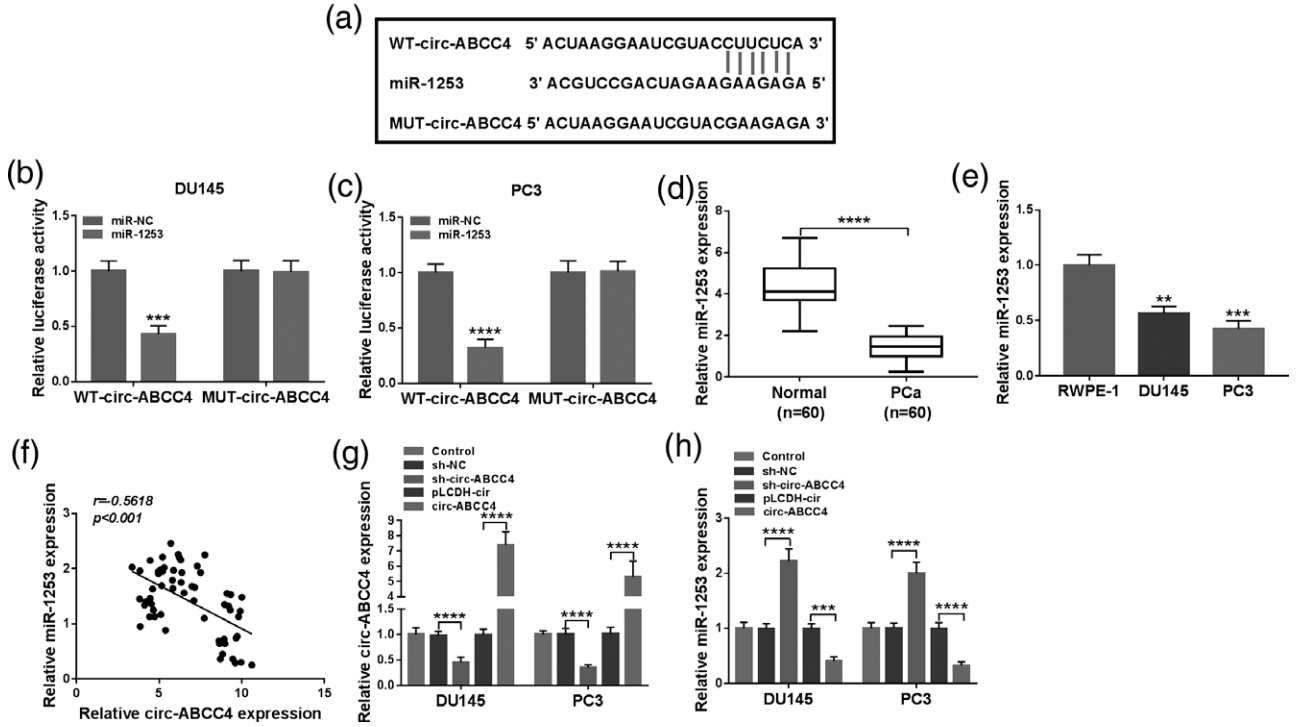
Circ-ABCC4 interference contributes to irradiation-induced suppressive effect in xenografts growth *in vivo*

Circ-ABCC4 depletion or irradiation alone blocked xenografts growth (Fig. 3a and b). Circ-ABCC4 silencing further aggravated irradiation-mediated suppressive influence in PCa tumor growth *in vivo* (Fig. 3a and b). RT-qPCR revealed that circ-ABCC4 was successfully silenced (Fig. 3c). In addition, we determined the protein abundance of proliferation-associated indicators (Ki67 and CyclinD1) and metastasis-related marker (MMP9) in xenografts through IHC assay. Circ-ABCC4 silencing alone or irradiation exposure alone reduced Ki67, CyclinD1, and MMP9 abundance in xenograft specimens (Fig. 3d). Furthermore, combined treatment of circ-ABCC4 knockdown and irradiation exposure further reduced Ki67, CyclinD1, and MMP9 enrichment (Fig. 3d). Overall, circ-ABCC4 knockdown aggravated the inhibitory effect of irradiation on the growth of xenografts.

MicroRNA-1253 is a downstream molecule of circ-ABCC4

The supposed binding sites of miR-1253 and circ-ABCC4 foretold by circinteractome are presented in Fig. 4a. Luciferase intensity was conspicuously declined in WT-circ-ABCC4 group by miR-1253 accumulation (Fig. 4b and c), proving that miR-1253 bound

Fig. 4



miR-1253 is a downstream molecule of circ-ABCC4. (a) The supposed binding sequence of miR-1253 and circ-ABCC4 foretold by circinteractome database was presented. (b and c) The target relation of miR-1253 and circ-ABCC4 was validated via a dual-luciferase reporter assay. (d) miR-1253 abundance in PCa and normal tissue specimens ($n=60$) was examined via RT-qPCR. (e) RT-qPCR was applied to assess miR-1253 enrichment. (f) The linear correlation between miR-1253 and circ-ABCC4 abundance was evaluated via Spearman's correlation coefficient. (g and h) Circ-ABCC4 and miR-1253 abundance in DU145 and PC3 cells were examined via RT-qPCR. $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$. miR-1253, microRNA-1253; PCa, prostate cancer.

to circ-ABCC4. miR-1253 enrichment was markedly declined in PCa tissue specimens (Fig. 4d). miR-1253 enrichment was declined in PCa cell lines (Fig. 4e). miR-1253 abundance in PCa tissue specimens was negatively correlated with circ-ABCC4 abundance (Fig. 4f). The efficiencies of sh-circ-ABCC4 and circ-ABCC4 were validated by RT-qPCR (Fig. 4g). Circ-ABCC4 could negatively modulate miR-1253 abundance in PCa cells (Fig. 4h). Overall, miR-1253 was a downstream molecule of circ-ABCC4.

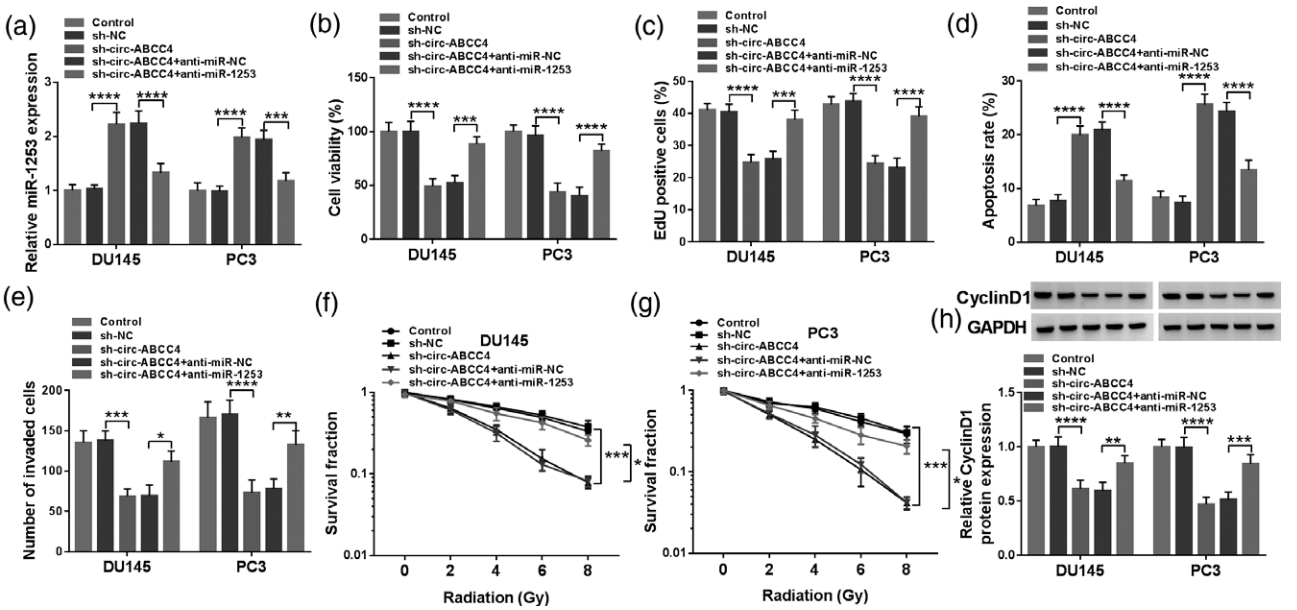
Circ-ABCC4 accelerates prostate cancer progression through sponging microRNA-1253 *in vitro*

Given the results that circ-ABCC4 depletion enhanced the expression of miR-1253, and circ-ABCC4 interference suppressed PCa progression, we aimed to investigate if circ-ABCC4 knockdown-mediated influences in PCa cells were related to miR-1253. Sh-circ-ABCC4-induced upregulation of miR-1253 was offset by silencing miR-1253 (Fig. 5a). miR-1253 knockdown largely rescued cell viability in circ-ABCC4-silenced PCa cells (Fig. 5b). Circ-ABCC4 interference suppressed PCa cell proliferation ability, which was recovered by knocking down miR-1253 (Fig. 5c). Circ-ABCC4 interference-induced cell apoptosis was partly neutralized by knocking down miR-1253 (Fig. 5d). Invaded cell number was decreased by knocking down circ-ABCC4, and cell invasion ability was partly rescued in co-transfected group (Fig. 5e). Circ-ABCC4 knockdown elevated PCa cell radiosensitivity, and miR-1253 depletion partly recovered radioresistance of PCa cells (Fig. 5f and g). Circ-ABCC4 silencing-induced inhibitory effect on CyclinD1 was largely offset by down-regulating miR-1253 (Fig. 5h). Taken together, circ-ABCC4 absence hampered PCa cell progression largely by enhancing miR-1253 abundance.

SOX4 is a downstream molecule of microRNA-1253

miRNAs could bind to mRNAs and negatively regulate the expression of mRNAs [19]. To deeply understand the working mechanism of miR-1253 in PCa progression, targetscan was adopted to foretell downstream partners of miR-1253. The supposed interacted sequence of miR-1253 and *SOX4* predicted by targetscan was presented (Fig. 6a). The luciferase intensity declined in miR-1253 and SOX4 3′UTR-WT co-transfected group (Fig. 6b and c), suggesting that miR-1253 bound to SOX4 3′UTR via the predicted complementary sequence. *SOX4* mRNA abundance was notably

Fig. 5



Circ-ABCC4 accelerates PCa progression through sponging miR-1253 *in vitro*. (a) miR-1253 level in transfected PCa cells was determined via RT-qPCR. (b) Cell viability was examined via MTT assay. (c) Cell proliferation was analyzed via EdU assay. (d) Cell apoptosis was evaluated via flow cytometry. (e) Cell invasion was examined via transwell invasion assay. (f and g) Colony formation assay was implemented to evaluate PCa cell sensitivity to irradiation. (h) The protein level of CyclinD1 was analyzed via western blot assay. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. miR-1253, microRNA-1253; EdU, 5-ethynyl-2'-deoxyuridine; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide; PCa, prostate cancer.

increased in PCa tissue specimens (Fig. 6d). The Cancer Genome Atlas and gene expression profiling interactive analysis databases revealed that circ-ABCC4 enrichment was increased in prostate adenocarcinoma tissue specimens (Fig. 6e and f). SOX4 protein enrichment was enhanced in PCa tissue specimens (Fig. 6g). A marked upregulation in SOX4 protein abundance was found in DU145 and PC3 cells (Fig. 6h). *SOX4* abundance in PCa tissue specimens was negatively correlated with miR-1253 enrichment (Fig. 6i). Overall, *SOX4* was a downstream molecule of miR-1253.

SOX4 overexpression overturns microRNA-1253-mediated impacts

We confirmed overexpression efficiency of miR-1253 (Fig. 7a). miR-1253 overexpression reduced the expression of SOX4 in PCa cells, and we co-transfected PCa cells with miR-1253 and SOX4 to rescue the expression of SOX4 (Fig. 7b). Cell viability was decreased with the accumulation of miR-1253, and cell viability was largely regained by *SOX4* overexpression (Fig. 7c). miR-1253 blocked PCa cell proliferation, which was largely rescued by *SOX4* overexpression (Fig. 7d). miR-1253 promoted PCa cell apoptosis, and *SOX4* plasmid addition hampered cell apoptosis (Fig. 7e). *SOX4* overexpression largely recovered the invasion capacity in miR-1253-overexpressed PCa cells (Fig. 7f). miR-1253 accumulation hampered PCa cell radioresistance, and the radioresistance was partly restored in co-transfected group (Fig. 7*g* and h). miR-1253 overexpression decreased CyclinD1 enrichment, and CyclinD1 was largely rescued by the introduction of *SOX4* plasmid (Fig. 7i). These results suggested that miR-1253 was restrained cell viability, proliferation, invasion, and radioresistance whereas promoted cell apoptosis of PCa cells largely by decreasing *SOX4* enrichment.

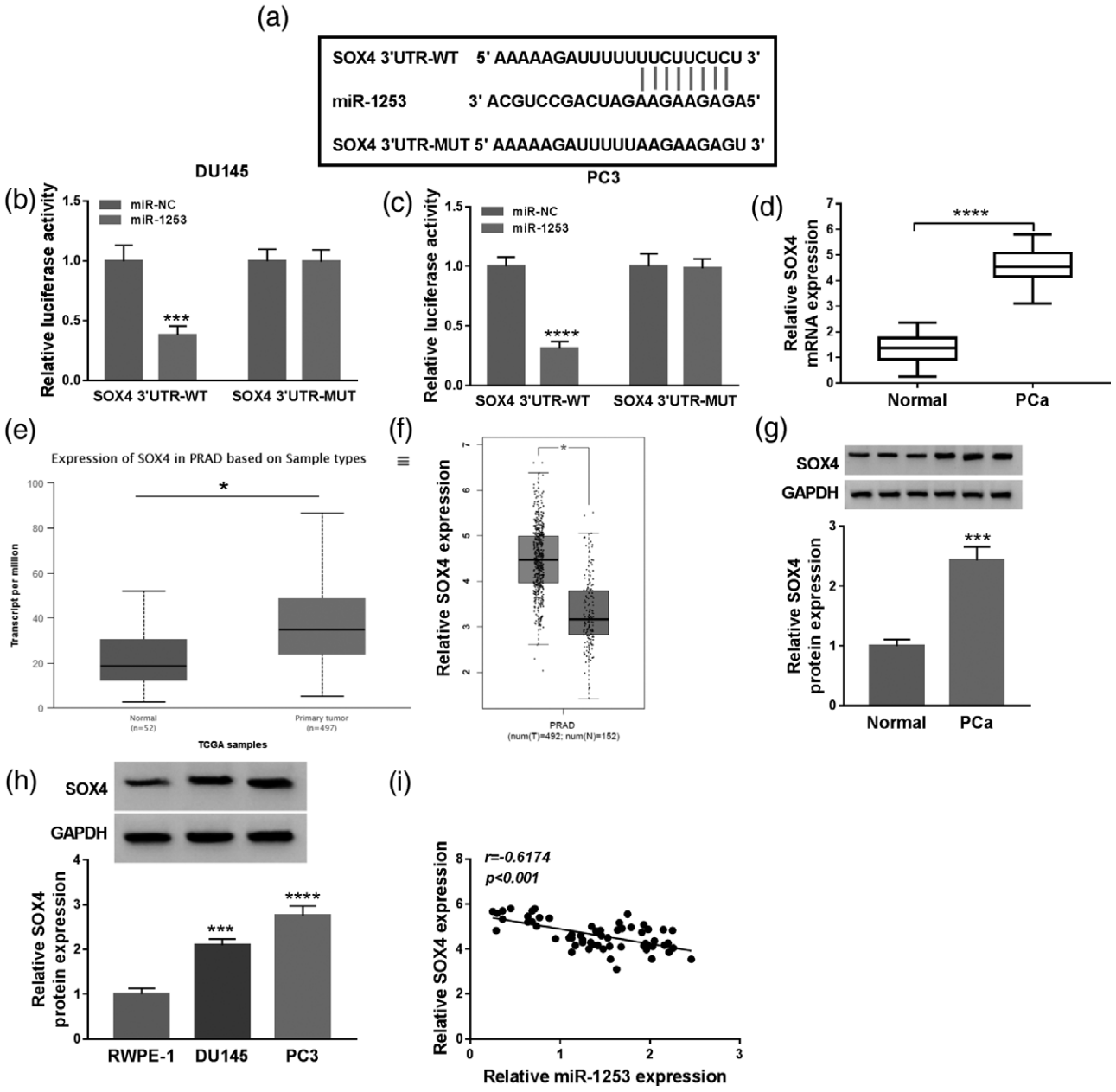
Circ-ABCC4 absorbs microRNA-1253 to enhance SOX4 abundance

Circ-ABCC4 knockdown declined the protein abundance of SOX4, while SOX4 was recovered by anti-miR-1253 (Fig. 8a and b). These findings proved that circ-ABCC4 enhanced SOX4 level through sponging miR-1253 in PCa cells.

Discussion

CircRNAs have shown pivotal regulatory roles in many malignancies [20,21]. For instance, CircRNA ITCH restrained PCa progression by enhancing *HOXB13* abundance via sequestering miR-17-5p [22]. CircRNA PSMC3 suppressed the proliferation of PCa cells by reducing *DGCR8* abundance [23]. Furthermore, circRNAs were also implicated in the modulation of cancer cell radioresistance. Wang *et al.* found that circ_0001313 silencing elevated the radiosensitivity of colon cancer cells by targeting miR-338-3p [24]. Circ-ABCC4 was

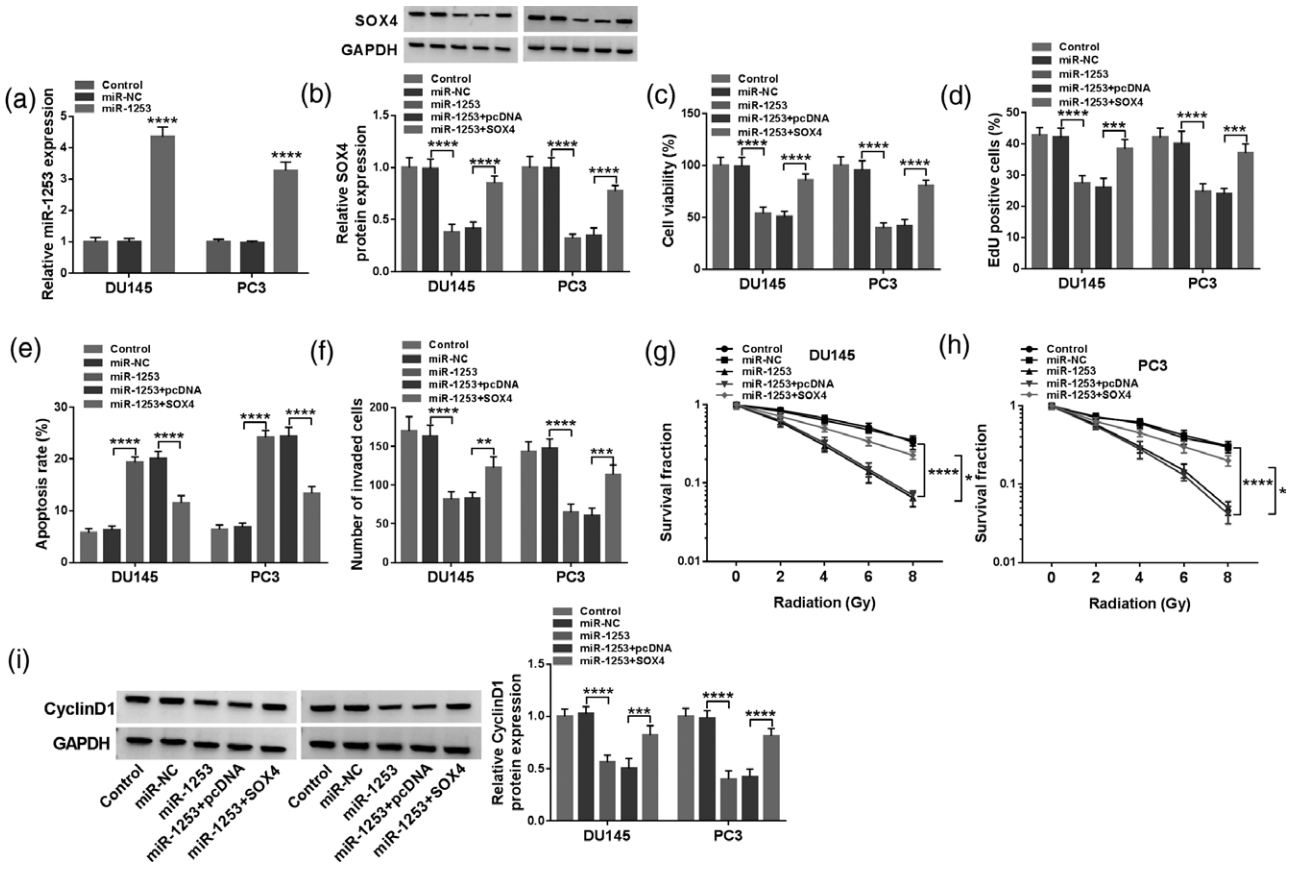
Fig. 6



SOX4 is a downstream molecule of miR-1253. (a) SOX4 was foretold to be a downstream partner of miR-1253 by targetscan. (b and c) The target relation of miR-1253 and SOX4 was verified via a dual-luciferase reporter assay. (d) SOX4 mRNA abundance in PCa tissue specimens was measured via RT-qPCR. (e) The expression of SOX4 mRNA in prostate adenocarcinoma (PRAD) tissues and normal tissues in TCGA database was shown. (f) SOX4 mRNA abundance in PRAD tissue specimens in GEPIA database was shown. (g) Western blot assay was employed to evaluate SOX4 abundance in PCa tissue specimens. (h) SOX4 protein enrichment in DU145, PC3, and RWPE-1 was examined via western blot assay. (i) Spearman's correlation coefficient was adopted to evaluate the linear relation of SOX4 and miR-1253. *$P<0.05$, ***$P<0.001$, ****$P<0.0001$. GEPIA, gene expression profiling interactive analysis; miR-1253, microRNA-1253; PCa, prostate cancer; SOX4, SRY-box transcription factor 4; TCGA, The Cancer Genome Atlas.

reported to function as an oncogene in PCa [10] and lung adenocarcinoma [25]. Huang *et al.* demonstrated that circ-ABCC4 absorbs miR-1182 to elevate *FOXP4* abundance to accelerate the progression of PCa [10]. Liu *et al.* found that circ-ABCC4 aggravated lung adenocarcinoma progression by modulating miR-3186-3p/*TNRC6B* cascade [25]. Here, circ-ABCC4 abundance was elevated in PCa. Moreover, circ-ABCC4 enrichment was closely associated with the clinicopathologic feature of PCa patients, suggesting that circ-ABCC4 might be a novel prognostic

Fig. 7



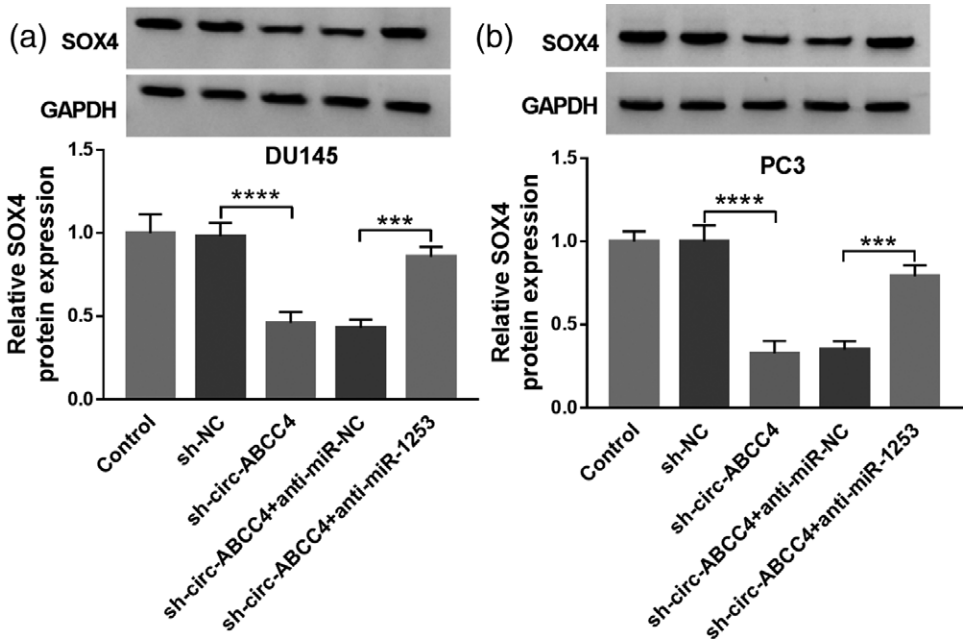
SOX4 accumulation offsets miR-1253-induced influences in PCa cells. (a) DU145 and PC3 cells were introduced with miR-NC or miR-1253, and the overexpression efficiency of miR-1253 mimics was assessed via RT-qPCR. (b–i) DU145 and PC3 cells were introduced with miR-NC, miR-1253, miR-1253 + pcDNA, or miR-1253 + SOX4. (b) Western blot assay was adopted to evaluate SOX4 abundance. (c) Cell viability was analyzed via MTT assay. (d) EdU assay was conducted to evaluate PCa cell proliferation. (e) The apoptosis rate was analyzed by flow cytometry. (f) Transwell invasion assay was adopted to assess cell invasion. (g and h) Colony formation assay was performed to evaluate PCa cell radioresistance. (i) CyclinD1 was examined via western blot. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$. EdU, 5-ethynyl-2'-deoxyuridine; miR-1253, microRNA-1253; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide; PCa, prostate cancer; SOX4, SRY-box transcription factor 4; 3′UTR, 3′ untranslated region.

marker. Circ-ABCC4 depletion restrained PCa cell viability, proliferation, invasion, and radioresistance and elevated cell apoptosis rate. Subsequently, circ-ABCC4 role in PCa tumor growth with the treatment of irradiation was explored via a tumor xenograft experiment. Circ-ABCC4 interference contributed to irradiation-mediated suppressive influence in xenografts growth *in vivo*.

miR-1253 was identified as a downstream molecule of circ-ABCC4. miR-1253 blocked the development of many malignancies. Huang *et al.* claimed that circNASP aggravated the malignant progression of osteosarcoma through sponging and suppressing miR-1253 to enhance the abundance of *FOXF1* [26]. miR-1253 restrained the progression of medulloblastoma by targeting *CDK6* and *CD276* [27]. miR-1253 hampered NSCLC development by targeting *WNT5A* [28]. As for PCa, miR-1253 acted as the target of FOXC2-AS1 to decrease PCa cell malignant potential through regulating *EZH2* [13]. miR-1253 expression was markedly reduced in PCa. Through compensation assays, we observed that circ-ABCC4 interference-mediated anti-tumor impacts were partly counteracted by anti-miR-1253, suggesting that circ-ABCC4 knockdown hindered PCa progression by enhancing miR-1253 abundance.

miRNAs reduced the expression of target mRNAs via binding to them [29]. For instance, miR-9-5p silencing restrained PCa development by targeting *StarD13* [30]. miR-519d hindered PCa cell biological properties by targeting *NRBP1* [31]. *SOX4* was validated as a downstream molecule of miR-1253. Feng *et al.* found that miR-19a-3p blocked PCa cell motility by modulating *SOX4* [17]. Wang *et al.* demonstrated that *SOX4* high enrichment was associated with the dismal outcome of PCa cases and *SOX4* accelerated epithelial–mesenchymal transition of

Fig. 8



Circ-ABCC4 sequesters miR-1253 to enhance SOX4 enrichment. (a and b) Cells were introduced with sh-NC, sh-circ-ABCC4, sh-circ-ABCC4 + anti-miR-NC or sh-circ-ABCC4 + anti-miR-1253. SOX4 abundance in PCa cells was determined via western blot assay. ***$P<0.001$, ****$P<0.0001$. miR-1253, microRNA-1253; PCa, prostate cancer; PRAD, prostate adenocarcinoma; SOX4, SRY-box transcription factor 4.

PCa cells [15]. *SOX4* was also reported to regulate cancer cell radiosensitivity. miR-130a was found to elevate the radiosensitivity of rectal cancer cells by targeting *SOX4* [18]. *SOX4* expression was significantly enhanced in PCa. Through performing rescue experiments, we observed that miR-1253 accumulation decreased PCa cell malignant potential largely by down-regulating *SOX4*. Circ-ABCC4 could sequester miR-1253 to enhance *SOX4* abundance in PCa cells.

In summary, circ-ABCC4 facilitated PCa cell viability, proliferation, invasion, and radioresistance and hindered cell apoptosis by sequestering miR-1253 and enhancing *SOX4* abundance. Therefore, high expression of circ-ABCC4 and *SOX4* might be novel markers for radiotherapy non-responders, and circ-ABCC4/miR-1253/*SOX4* axis might be a new target for PCa treatment.

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Conflicts of interest

There are no conflicts of interest.

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