Circ-ABCC4 contributes to prostate cancer progression and radioresistance by mediating miR-1253/SOX4 cascade
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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 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

Keywords: circ-ABCC4, irradiation, microRNA-1253, prostate cancer, SRY-box transcription factor 4

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/b-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...
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 quantitate the abundance of circ-ABCC4, miR-1253, and SOX4 mRNA. The abundance was analyzed by $2^{-\Delta\Delta C_t}$. 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 2h. 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 volume = length × width^2 × 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       | GGAGGAGAAGAAGAAGAATCA    |
| SOX4      | Human   | GCACCTGGCAGCCTGCTGCTT    | GACACGGGATATTGCAGGAGA    |
| U6        | Human   | GCTTGCAGCAGCAGCATATTAAAAAT | GCCTTACGAATTTGCGTGTGATC |
| GAPDH     | Human   | CCTGTTCGACAGTCAGCCG      | GAGAACAGTGAGCGGCTAGT     |

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 200g 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 2h. 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 patients with positive lymph node metastasis or not was shown (Fig. 1d). Percent survival of PCa cases with high or low abundance of circ-ABCC4 in five years was shown, *P* = 0.002, ***P* < 0.001, ****P* < 0.0001.
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 PCa cell viability, proliferation, invasion, and radioresistance whereas induces 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.

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
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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). 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
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. 6a), suggesting that miR-1253 bound to SOX4 3’UTR via the predicted complementary sequence. SOX4 mRNA abundance was notably

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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. 7g 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 PSMG3 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...
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
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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 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 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.

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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.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1. Etzioni R, Urban N, Ramsey S, McIntosh M, Schwartz S, Reid B, et al. The case for early detection. Nat Rev Cancer 2003; 3:243–252.
2. Poorthuis MHF, Verhoef RWM, van Moorselaar RJA, de Reijke TM. Second-line therapy in patients with metastatic castration-resistant prostate cancer with progression after or under docetaxel: a systematic review of nine randomized controlled trials. Semin Oncol 2017; 44:358–371.
3. Chen R, Sheng L, Zhang HJ, Ji M, Qian WQ. miR-15b-5p facilitates the tumorigenicity by targeting RECK and predicts tumour recurrence in prostate cancer. J Cell Mol Med 2018; 22:1855–1863.
4. Nigro JM, Cho KR, Fearon ER, Kern SE, Ruppert JM, Oliner JD, et al. Scrambled exons. Cell 1991; 64:607–613.
5. Rybak-Wolf A, Stottmeister C, Glazier P, Jens M, Pino N, Giusti S, et al. Circular RNAs in the mammalian brain are highly abundant, conserved, and dynamically expressed. Mol Cell 2015; 58:870–885.
6. Huang S, Yang B, Chen BJ, Blain N, Ueberham U, Arendt T, Janitz M. The emerging role of circular RNAs in transcriptome regulation. Genomics 2017; 109:401–407.
7. Chen LL. The biogenesis and emerging roles of circular RNAs. Nat Rev Mol Cell Biol 2016; 17:205–211.
8. Kristensen LS, Hansen TB, Vere MT, Kjemsa J. Circular RNAs in cancer: opportunities and challenges in the field. Oncogene 2018; 37:555–565.
9. Song Z, Zhuo Z, Ma Z, Hou C, Chen G, Xu G. Hsa_Circ_0001206 is downregulated and inhibits cell proliferation, migration and invasion in prostate cancer. Artif Cells Nanomed Biotechnol 2019; 47:2449–2464.
10. Huang C, Deng H, Wang Y, Jiang H, Xu R, Zhu X, et al. Circular RNA circABCC4 as the ceRNA of miR-1182 facilitates prostate cancer progression by promoting FOXP4 expression. J Cell Mol Med 2019; 23:6112–6119.
11. Wang D, Lu G, Shao Y, Xu D. MiR-182 promotes prostate cancer progression through activating Wnt/β-catenin signal pathway. Biomed Pharmacother 2018; 99:334–339.
12. Wang Y, Lieberman R, Pan J, Zhang Q, Du M, Zhang P, et al. miR-375 induces docetaxel resistance in prostate cancer by targeting SEC23A and YAP1. Mol Cancer 2016; 15:70.
13. Chen Y, Gu M, Liu C, Wan X, Shi Q, Chen Q, Wang Z. Long noncoding RNA FOXC2-AS1 facilitates the proliferation and progression of prostate cancer via targeting miR-1253/EZH2. Gene 2019; 666:37–42.
14. Tiwari N, Tiwari VK, Waldmeier L, Balwierz PJ, Arnold P, Pachkov M, et al. Sox4 is a master regulator of epithelial-mesenchymal transition by controlling Ezh2 expression and epigenetic reprogramming. Cancer Cell 2013; 23:768–783.
15. Wang L, Zhang J, Yang X, Chang YW, Qi M, Zhou Z, et al. SOX4 is associated with poor prognosis in prostate cancer and promotes epithelial-mesenchymal transition in vitro. Prostate Cancer Prostatic Dis 2013; 16:301–307.
16. Liu P, Ramachandran S, Ali Seyed M, Scharer CD, Laycock N, Dalton WB, et al. Sex-determining region Y box 4 is a transforming oncogene in human prostate cancer cells. Cancer Res 2006; 66:4011–4019.
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17 Feng YG, Zhao JF, Xiao L, Rao WY, Ran C, Xiao YH. MicroRNA-19a-3p suppresses invasion and metastasis of prostate cancer via inhibiting SOX4. *Eur Rev Med Pharmacol Sci* 2018; 22:6245–6251.

18 Ha Thi HT, Kim HY, Kim YM, Hong S. MicroRNA-130a modulates a radiosensitivity of rectal cancer by targeting SOX4. *Neoplasia* 2019; 21:882–892.

19 Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; 75:843–854.

20 Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P, Wu M. CircRNA: functions and properties of a novel potential biomarker for cancer. *Mol Cancer* 2017; 16:94.

21 Zhang HD, Jiang LH, Sun DW, Hou JC, Ji ZL. CircRNA: a novel type of biomarker for cancer. *Breast Cancer* 2018; 25:1–2.

22 Wang X, Wang R, Wu Z, Bai P. Circular RNA ITCH suppressed prostate cancer progression by increasing HOXB13 expression via spongy miR-17-5p. *Cancer Cell Int* 2019; 19:328.

23 Dong JS, Wu B, Chen XH. Circ PSMC3 inhibits prostate cancer cell proliferation by downregulating DGCR8. *Eur Rev Med Pharmacol Sci* 2020; 24:2264–2270.

24 Wang L, Peng X, Lu X, Wei Q, Chen M, Liu L. Inhibition of hsa_circ_0001313 (circCCDC66) induction enhances the radio-sensitivity of colon cancer cells via tumor suppressor miR-338-3p: Effects of cicr_0001313 on colon cancer radio-sensitivity. *Pathol Res Pract* 2019; 215:689–696.

25 Liu M, Wang P, Sui X, Ding F, Liu L, Gao Z, Cheng Z. Circular RNA circABCC4 regulates lung adenocarcinoma progression via miR-3186-3p/TNRC6B axis. *J CellBiochem* 2020; 121:4226–4238.

26 Huang L, Chen M, Pan J, Yu W. Circular RNA cicrNASP modulates the malignant behaviors in osteosarcoma via miR-1253/FOXF1 pathway. *Biochem Biophys Res Commun* 2018; 500:511–517.

27 Kanchan RK, Perumal N, Ari P, Chirravuri Venkata R, Thapa I, Klinkebiel DL, et al. MiR-1253 exerts tumor-suppressive effects in medulloblastoma via inhibition of CDK6 and CD276 (B7-H3). *Brain Pathol* 2020; 30:732–745.

28 Liu M, Zhang Y, Zhang J, Cai H, Zhang C, Yang Z, et al. MicroRNA-1253 suppresses cell proliferation and invasion of non-small-cell lung carcinoma by targeting WNT5A. *Cell Death Dis* 2018; 9:189.

29 Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem* 2010; 79:351–379.

30 Chen L, Hu W, Li G, Guo Y, Wan Z, Yu J. Inhibition of miR-9-5p suppresses prostate cancer progress by targeting Star13. *Cell Mol Biol Lett* 2019; 24:20.

31 Yan CQ, Lu YH, Tang SM, Fan WX. MiR-519d inhibits prostate cancer cell proliferation, cycle and invasion via targeting NRBP1. *Eur Rev Med Pharmacol Sci* 2018; 22:2985–2990.