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ORIGINAL PAPER

Knockdown of long non-coding RNA PCAT1 in glioma stem cells promotes radiation sensitivity

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Abstract

This study aimed to investigate the function of glioma stem cells (GSCs) and the role of PCAT1. This study dissociated the differences between GSCs and glioma cells in terms of apoptosis rate and γH2AX positive cells levels after radiation. Microarray was carried out to detect that expressed PCAT1, and it was testified by RT-qPCR. After transfection, GSCs were used to investigate the influence of PCAT1 on radiation sensitivity. Sphere-formation capability was first examined. Cell apoptosis rate after radiation of 0 Gy or 6 Gy was analyzed by flow cytometry, and the level of γH2AX positive cells after 6 Gy radiation were compared. CCK8 assay was used to investigate the cell proliferation and RT-qPCR was used to examine miR-129-5p and HMGB1 expression. GSCs exhibited great capability in sphere formation and lower expression in apoptosis and γH2AX positive cells rates after 6 Gy radiation. PCAT1 had higher expression in GSCs. PCAT1 knockdown restrained the sphere-formation ability, increased the apoptosis rate and DNA damage under the treatment of radiation. Moreover, knockdown of PCAT1 inhibited the cell proliferation. In addition, silencing PCAT1 could increase the expression of miR-129-5p and decrease the expression of HMGB1. PCAT1 was overexpressed in GSCs and played a facilitating role in radiation resistance.

Keywords Glioma · GSC · PCAT1 · γH2AX · Radiation

Introduction

Glioma is reported to be one of the most malignant brain tumors in the central nervous system [1]. According to World Health Organization (WHO) classification, glioma can be identified into four grades: glioma of grade I–II has relatively acceptable prognosis, while glioma of grade III–IV, such as glioblastoma (GBM), exhibited significantly poor prognosis [2]. The average survival of GBM patients was 14.6 months after a full set of treatment procedures of aggressive surgery, radiation therapy and chemotherapy [3]. This dismal outcome was associated with inefficiency of surgical resection caused by the invasive growth of glioma cells, which made tumor and normal brain tissues overlap with each other [4]. Radioresistance was another factor contributing to this low survival rate of malignant glioma [5].

Recently, numerous studies have demonstrated that there exists a subpopulation of cells in various cancers which displays the characteristics of stem cells (self-renewal and multipotency) and may play a critical role in tumor propagation, radioresistance, chemo-resistance, and recurrence [6]. Glioma stem-like cells (GSCs), one form of this kind of cancer stem cells identified in glioma, were found to grow non-adherent spheres in serum-free medium, showing the capability of tumor formation [7]. It was suggested in previous studies that GSCs could enhance resistance to radiation [6, 8]. Signaling pathways including NOTCH, Wnt/β-catenin, Hedgehog, STAT3, and PI3K/AKT/mTOR were reported by many researchers to regulate the GSCs radioresistance [5]. The capacity to activate DNA damage checkpoint response and increase DNA repair capacity of GSCs was proved to

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enhance the radioresistance [9]. This study would further explore the underlying genomic mechanism in GSC-associated radioresistance from the point of lncRNAs to provide inspiration to the development of glioma treatment.

Long non-coding RNAs (lncRNAs, ~200 nt) are a class of RNAs that do not encode proteins [10]. Mounting evidences have testified that lncRNAs are involved in epigenetic regulation, gene transcription, mRNA processing and gene translation [11]. Besides, LncRNAs were reported to be ubiquitously dysregulated in tumor cells and have crucial regulatory roles in the malignant progression of tumor cells, such as proliferation, migration and invasion, and apoptosis [12]. They have also been discovered to be highly expressed in the brain compared to other regions of human body. In the field of glioma studies, some lncRNAs were proved to influence glioma cells and GSCs. There also existed a close relationship between certain lncRNAs and the oncogenesis, differentiation, progression, recurrence and stem-like characteristics in glioma [13]. But how could certain lncRNAs regulate GSCs radioresistance remained elusive.

PCAT1 (Prostate Cancer-Associated Transcript 1) as one of the lncRNAs was proven in many studies to play a critical role in various cancers and tumors. It was proved that PCAT1 upregulation was associated with TNM (tumor node metastasis) stage, metastasis and poor overall survival in HCC (hepatocellular carcinoma) [14], colorectal cancer (CRC) [15], and esophageal squamous cell carcinoma (ESCC) [16]. PCAT1 also played a role in the drug resistance of CRC cells [17]. In glioma research field, literatures focusing on the function of PCAT1 were limited. Though Balci et al. [18] demonstrated that PCAT1 showed lower expression in cancer cells than in GSCs, further efforts were required to obtain a better understanding of the underlying mechanism of PCAT1 on GSCs and to improve current therapies.

In this study, we aimed to investigate the function of glioma stem cells in radiation insensitivity and the role of PCAT1 in this process, finding that PCAT1 in GSCs played a facilitating role in radiation resistance. Therefore, future therapy could target PCAT1 in GSCs to increase the efficacy of radiation in glioma treatment.

Materials and methods

Human glioma tissue specimens and patient information

Primary glioma tissue specimens were obtained from patients through neurosurgical resection at Heilongjiang Provincial Hospital. All procedures were carried out in accordance with the ethical regulations of Heilongjiang Provincial Hospital. Written consents were obtained from all patients in accordance with related rules and regulations.

Dissociation and culture of glioma stem cells

The glioma stem cells were sorted out from total glioma cells (separating from the same fresh glioma sample) by flow cytometry after using CD133 surface marker staining. Briefly, glioblastoma tumor samples were disaggregated in 1% PBS solution containing 0.3% collagenase-I to obtain tumor cells. Cells were collected via centrifugation and then were resuspended in Dulbecco's modified Eagle's Medium. Subsequently, cells were stained with a FITC-conjugated anti-human CD133 antibody. Then, the isolation and enrichment of CD133+ human glioma stem cells were achieved using a flow cytometer. Then, the glioma cancer cells were cultured in DMEM/F-12 medium (Life Technologies Corporation, Grand Island, NY, USA) in a nut shell free of serum. Basic fibroblast growth factor (bFGF, 20 ng/mL, Life Technologies Corporation, Carlsbad, CA, USA), epidermal growth factor (EGF, 20 ng/mL, Life Technologies Corporation, Gaithersburg, MD, USA) and 2% B27(Life Technologies Corporation, Grand Island, NY, USA) were supplemented. Under these conditions, the cancer cells grow as nonadherent spherical clusters of cells called mammospheres. Half of the media was changed every other day. Five days later, mammospheres were collected by centrifugation at 1000 rpm for 5 min and further plated in the new medium. The glioma stem cells mammospheres were cultured in serum-free medium under 5% CO_2 at 37 °C.

Sphere formation assay

The cells were transferred to a low-attachment 24-well flat plate (Prime surface, Sumitomo) in a serum-free DMEM containing 20 ng/mL bFGF (Life Technologies Corporation, Carlsbad, CA, USA), 10 µg/mL human insulin (CSTT), 100 µg/mL human transferrin (Roche) and 100 µg/mL BSA (Nacalai Tesque) and incubated at 37 °C in a 5% CO_2 incubator. 1.0×10^4 cells were seeded per well and cultured for 10 days. The spheres that were larger than 100 µm were counted.

RNA isolation

Total RNA was extracted from glioma cancer cells and stem cells using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. The concentration and quality of RNA was assessed via NanoDrop ND-1000 spectrophotometry (Thermo Scientific) and denatured for agarose gel electrophoresis. After removal of rRNA, purified RNA was amplified and transcribed into fluorescent cRNA using

Agilent's Quick Amp Labeling protocol according to the manufacturer's protocol.

Microarray analysis and quantitative real-time PCR (RT-qPCR)

Microarray analysis was performed. Briefly, the labeled cRNAs were hybridized onto the 4 × 44 K Rat LncRNA Array (Arraystar, Rockville, MD) at 65 °C for 17 h. Hybridization images were collected using an Agilent Microarray Scanner G2565BA. Data were analyzed using Agilent Feature Extraction software. Further analysis was performed using the GeneSpring GX v11.5.1 software package (Agilent Technologies). Gene expression level was quantified through quantitative real-time PCR using the QuantiTect SYBR Green PCR kit (Qiagen, Canada) on the StepOnePlus Real-time PCR system (ThermoFisher Scientific, Canada). All primers were commercially obtained from Genepharma (Shanghai, China) as follows: PCAT1 (forward): 5′-TTGTGGAAGCCCCGCAAGGCCTGAA-3′; PCAT1 (reverse): 5′-TGTGGGGCCTGCACTGGCACTT-3′; CD133 (forward): 5′-CAGAGT ACA ACG CCA AAC CA-3′, CD133 (reverse): 5′-AAA TCA CGA TGA GGG TCA GC-3′; ABCG2 (forward): 5′-GACTGA AGG GCT ACT AAC C-3′. ABCG2 (reverse): 5′-TGCCCAGGACTC AATGCAACAG-3′; miR-129-5p (forward): 5′-GATCCG CAAGCCCAGACCGCAAAAAGTTTTTA-3′, miR-129-5p (reverse): 5′-AGCTTAAAAACTTTTTGCGGTCTGGGC TTGCG-3′; HMGB1 (forward): 5′-GCGGACAAGGCC CGTTA-3′; HMGB1 (reverse): 5′-AGAGGAAGAAGG CCGAAGGAA-3′; U6 (forward): 5′-TGGAACGCTTCA CGAATTTGCG-3′, U6 (reverse): 5′-GGAACGATACAG AGAAGATTAGC-3′; GAPDH (forward): 5′-GGAGCGAGA TCCCTCCAAAAT-3′. GAPDH (reverse): 5′-GGCTGTTGT CATACTTCTCATGG-3′. Expression levels were calculated using the comparative C_t method via StepOne Software, and relative expression levels normalized to GAPDH.

Cell transfection

Glioma stem cells were transfected with pcDNA3.1-PCAT1 to be upregulated or PCMV-shPCAT1 to be downregulated using Lipofectamine 2000 reagent (Life Technologies Corporation, Carlsbad, CA, USA). The transfected efficacy was evaluated by RT-qPCR, and the high transfection efficacy of these could sustain 7 days from 48 h post-transfection, thus 72 h post-transfection was considered as the harvest time in the subsequent experiments.

Radiation treatment

Cells were exposed to radiation at a dose rate of 0 Gy/min or 6 Gy/min with 160 kV photons at room temperature using a RS2000 Biological Research Irradiator that contained a linear accelerator (Rad Source Technologies Asia Limited, LA, USA).

Apoptosis detection

Apoptosis was detected by Annexin V-APC/7-AAD staining (SouthernBiotech, Birmingham, AL, USA). The cells were harvested and stained by allophycocyanin (APC) and 7-aminoactinomycin D (AAD) following the instructions of the manufacturer. Cells were analyzed by flow cytometry (FACScan, BD Biosciences, San Jose, CA, USA) and apoptotic rates were quantified using CELL Quest 3.0 software.

Cell proliferation assay

CCK8 assay was performed in this study for detecting cell proliferation. After transfection for 24 h, 2×10^3 cells were put into 96-well plates, and cultured. CCK8 reagent was added in cells for 24 h, 48 h, 72 h, and 96 h, respectively. And cells were incubated at 37 °C for 2 h before detection. Microplate reader (Bio-Tek, USA) was applied to detect the absorption of each well at 450 nm.

Immunofluorescence assay

Cells were plated on coverslips and fixed in 4% methanol-free formaldehyde in PBS for 30 min, permeabilized with 0.1% Triton X-100 in PBS for 5 min, blocked with 5% FBS in PBS for 10 min and washed. Incubation with primary antibody γH2AX (H2A histone family member X, Millipore, Billerica, MA, USA) diluted 1:100 in PBS-gelatin was performed overnight at 4 °C. Cells were washed four times and incubated with fluorescein-conjugated secondary antibody goat-anti-mouse antibody (1:200; DAKO, Glostrup, Denmark). Fluorescence was visualized with a Zeiss Axioskip microscope (HBO100W/Z), equipped with a Canon digital camera (Canon PowerShot A640, Canon Inc., Tokyo, Japan) as well as imaging software (Canon Utilities, ZoomBrowser Ex. 5.7, Canon Inc., Nort Ryde, Australia). The number of γH2AX positive cells was counted manually. Nuclei were counterstained with 4′6-diamidino-2-phenylindole (DAPI).

Statistical analysis

GraphPad prism 6.0 software was used for statistical analysis. All data are presented as the mean ± standard deviation (SD) from at least three independent replicates. Statistical analysis of data was performed using the Student's *t* test. Differences were considered to be statistically significant when $P < 0.05$.

Results

GSCs were relatively insensitive to radiation compared with glioma cancer cells

GSCs were suspected to be responsible for the failure of cancer therapies including radiation treatment, as they were prone to be unaffected and were able to recapitulate the tumor [19]. To investigate the relationship between GSCs and radiation efficacy, this study first dissociated stem cells from primary cancer cells and tested the stemness of them through sphere formation assy. After staining with CD133 surface marker, flow cytometry was used to obtain the GSCs in our study. And the CD133 positive rate of sorted GSCs was 92.4% (Fig. 1a). As shown in Fig. 1b, many spheres were formed in GSCs demonstrating the characteristics of stem cells. Meanwhile, the stem cell markers were also examined in cancer cell and stem cells, respectively. Data indicated that CD133 and ABCG2 (ATP-binding cassette subfamily G member 2) were highly expressed in stem cells (Fig. 1c). Additionally, immunofluorescence assay showed that the level of γH2AX positive cells in stem cells after 6 Gy radiation was considerably lower than that in cancer cells (Fig. 1d, e), reflecting that less DNA double-strand breaks (DSB) were produced and/or were subject to repair in stem cells. As previous studies have addressed that inadequate apoptotic responses might be a major contributor to radioresistance [20], we therefore investigated apoptosis condition after radiation treatment of 0 Gy or 6 Gy between glioma cancer cells and GSCs. In both groups, stem cells exhibited lower rate of apoptosis than cancer cells. Moreover, cancer cells after 6 Gy radiation showed significantly higher rate of apoptosis compared to cancer cells with 0 Gy radiation. While stem cells also showed increased apoptosis rate after radiation, the scale of change was considerably small compared with that of cancer cells (Fig. 1f). This indicated that stem cells were relatively insensitive to radiation in comparison with cancer cells. Taken together, it could be concluded that, compared with glioma cancer cells, GSCs were relatively insensitive to radiation, which might be the cause of inefficacy of radiation treatment against glioma.

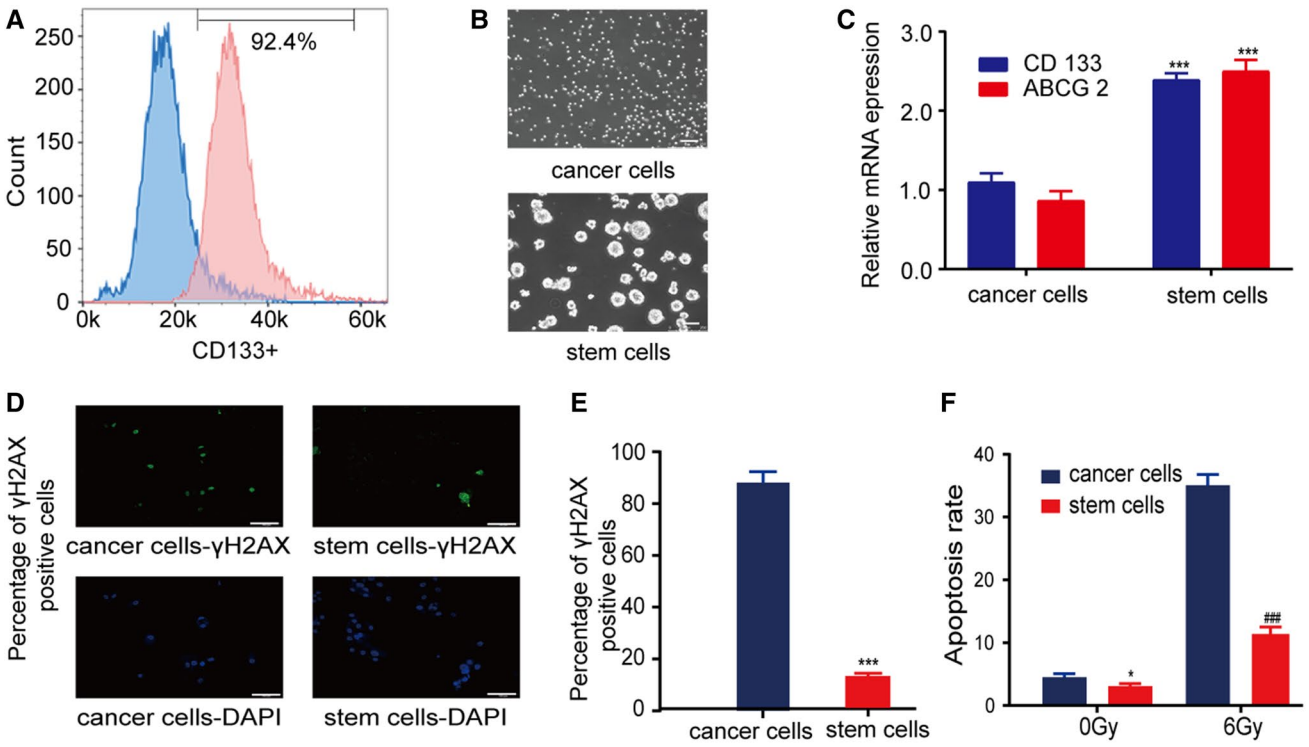

Fig. 1 GSCs were relatively insensitive to radiation compared with glioma cancer cells. **a** Flow cytometry was carried out to identify the sorted GSCs. **b** Sphere formation assay showing the number of spheres formed in GSCs after culturing for 10 days. Scale bar: 100 μm. **c** The stem cell markers (CD133 and ABCG2) were examined in cancer cell and stem cells by RT-qPCR, respectively (***$P<0.001$ vs. cancer cells group). **d** The immunofluorescent staining images of γH2AX positive cells in GSCs were observed under the fluorescence microscope. Scale bar: 100 μm. **e** Quantification of the γH2AX positive cells. Data were shown as mean ± SD of three experiments (***$P<0.001$ vs. cancer cells group). **f** Flow cytometry analysis of the apoptosis of GSCs after 0 Gy or 6 Gy radiation for 72 h (*$P<0.05$ and $^{\#\#}P<0.01$ vs. cancer cells group)

PCAT1 was upregulated in GSCs and involved radiation resistance

To identify the RNA that might induce the difference of response to radiation between glioma cancer cells and stem cells, microarray analysis was carried out to compare the lncRNA expression patterns of glioma cancer cells and stem cells. As shown in Fig. 2A, PCAT1 was notably over-expressed in stem cells. Later, a RT-qPCR analysis was performed to test the expression levels of PCAT1, proving that the relative expression of PCAT1 in stem cells was substantially higher than that in cancer cells (Fig. 2b). Thus, it could be concluded that PCAT1 was upregulated in GSCs and involved radiation resistance.

Knockdown of PCAT1 disrupts the maintenance of GSCs and increased the level of γH2AX positive cells

Having established the existence of PCAT1 in the response to radiation therapy, this study further examined deep correlation between PCAT1 and radiation efficacy. First, GSCs were transfected with pcDNA3.1-PCAT1 to be upregulated or pCMV-shPCAT1 to downregulated PCAT1 expression. The relative expression of PCAT1 after transfection was quantified by RT-qPCR and demonstrated in Fig. 2c. Then, sphere formation assay was employed to test the stemness among the four groups, presenting that the upregulation of PCAT1 considerably increased sphere formation while the knockdown of PCAT1 reduced this ability (Fig. 3a, b). CD133 and ABCG2 were the markers for the stem cells, we also detected the mRNA expression of the markers by RT-qPCR. In Fig. 3c, the expression of CD133 and ABCG2 were highly expressed in the pcDNA3.1-PCAT1 group and were significantly inhibited after PCAT1 knockdown. Regarding the level of γH2AX positive cells after radiation treatment, the enhancement of PCAT1 decreased the proportion of γH2AX positive cells dramatically, while the downregulation of PCAT1 augmented it (Fig. 3d, e). This reflected that knockdown of PCAT1 induced more DSB but inhibited DNA repair capability, making cells more sensitive to radiation.

The effects of PCAT1 on cell proliferation and apoptosis

In terms of apoptosis rate, in both 0 Gy-treated and 6 Gy-treated groups, upregulation of PCAT1 decreased the apoptosis cells while downregulation of PCAT1 enhanced cell apoptosis. Moreover, the negative influence of PCAT1 on cell apoptosis was more significant after 6 Gy radiation (Fig. 4a). To investigate the influence on cell proliferation

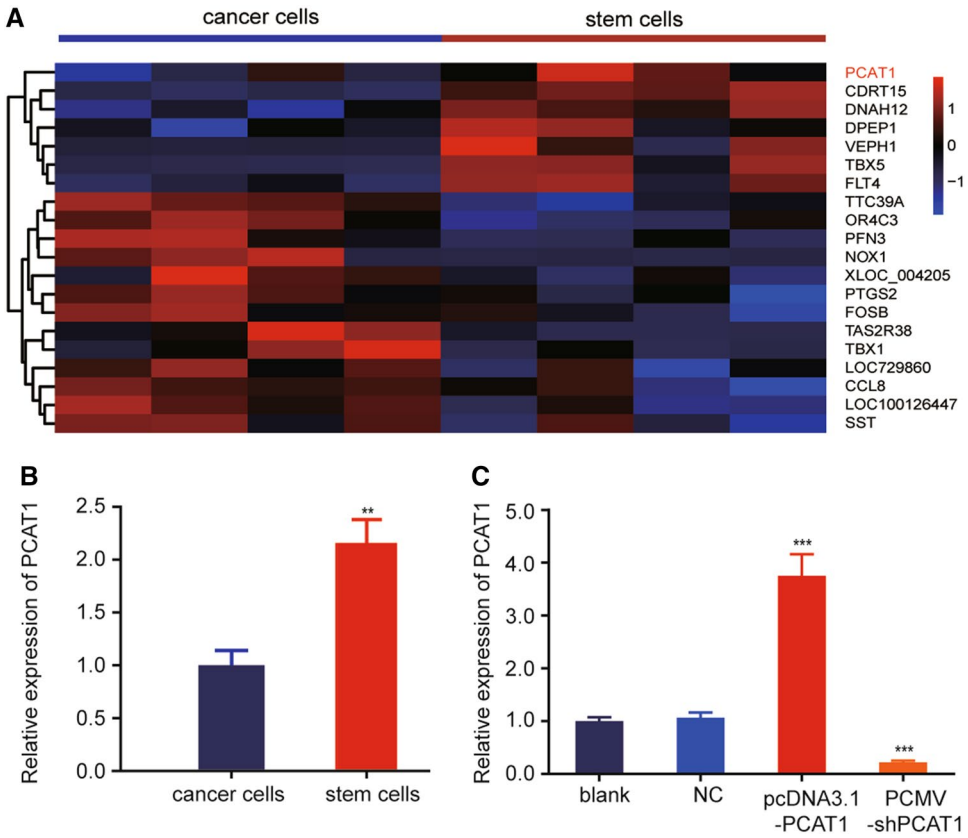

Fig. 2 Expression levels of PCAT1. **a** Heatmap showing lncRNA expression levels in stem cells and cancer cells examined by human lncRNA microarray, with red color indicating high relative expression, and blue low relative expression. **b** RT-qPCR analysis of relative expression levels of PCAT1 in stem cells and cancer cells (**$P < 0.01$vs. cancer cells group). **c** RT-qPCR analysis of relative expression levels of PCAT1 in GSCs, NC and PCAT1 after transfection with pcDNA3.1-PCAT1 or PCMV-shPCAT1 (***$P < 0.01$, compared to NC group)

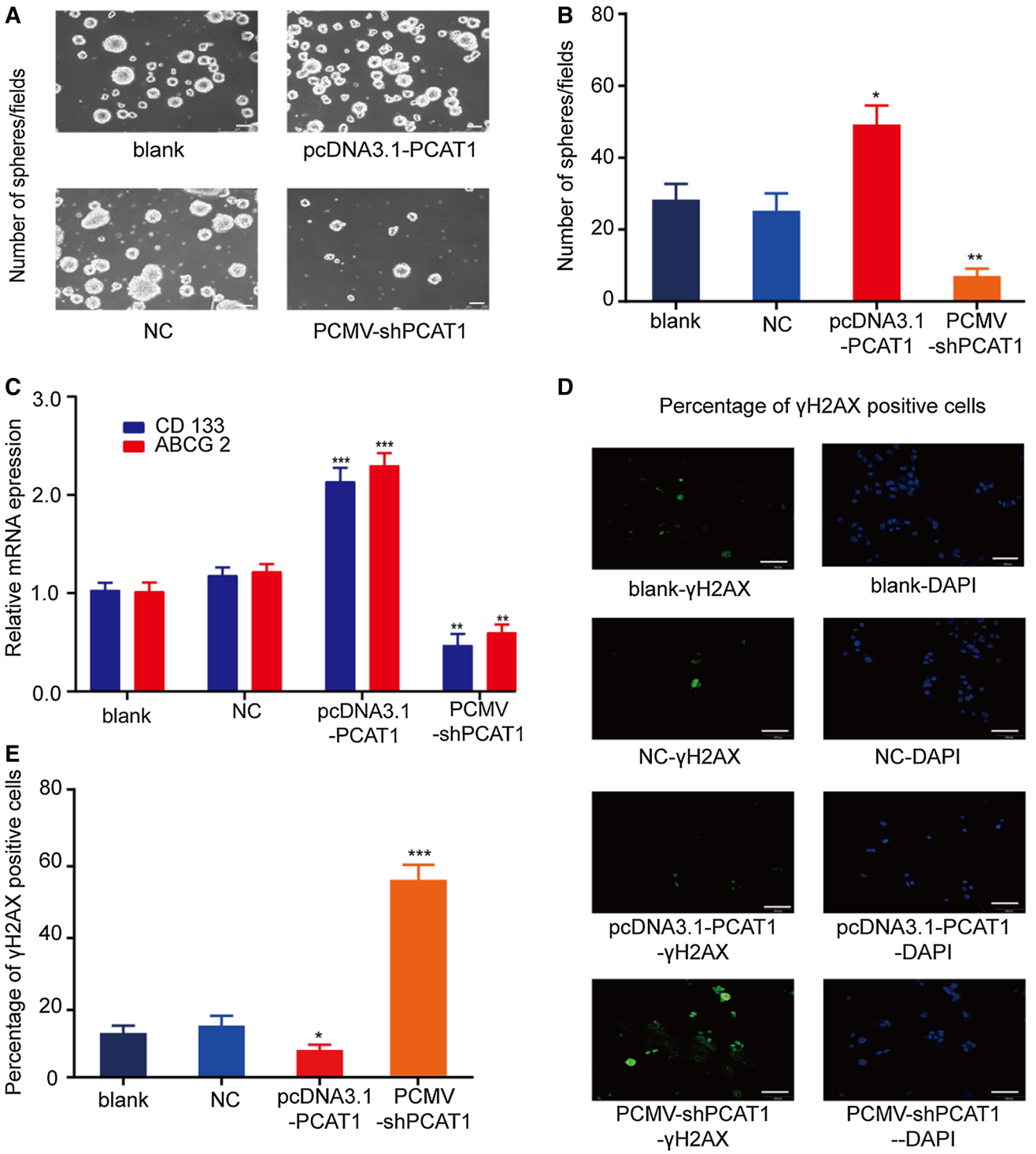

Fig. 3 The influence of PCAT1 on GSCs stemness and γH2AX positive cells. **a** Sphere formation assay of blank, NC, pcDNA3.1-PCAT1 and PCMV-shPCAT1 groups. Scale bar: 100 μm. **b** Quantification of the number of spheres formed in blank, NC, pcDNA3.1-PCAT1 and PCMV-shPCAT1 groups (*$P<0.05$, **$P<0.01$ vs. NC group). **c** The markers of GSCs were detected by RT-qPCR, including CD133 and ABCG2 (**$P<0.05$, ***$P<0.01$ vs. NC group). **d** The immunofluorescent staining of γH2AX positive cells and DAPI-counterstained cells in blank, NC, pcDNA3.1-PCAT1 and PCMV-shPCAT1 groups were observed under the fluorescence microscope. Scale bar: 100 μm. **e** Quantification of the proportion of γH2AX-positive cells in four groups (*$P<0.05$, ***$P<0.001$ vs. NC group)

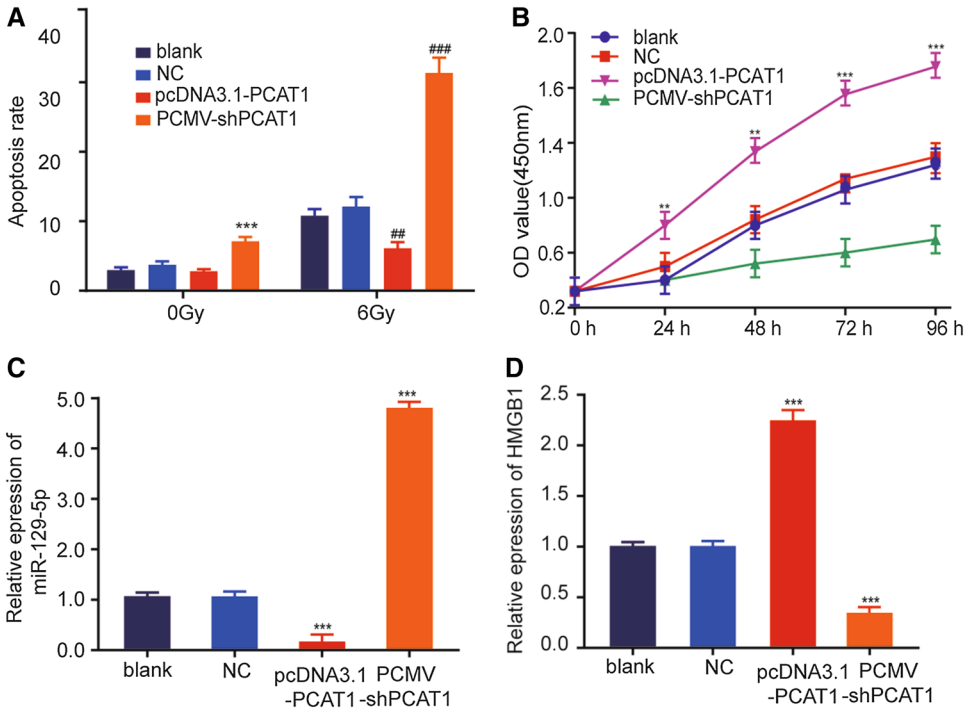

Fig. 4 The influence of PCAT1 on cell proliferation and apoptosis. **a** Flow cytometry analysis of the apoptosis of cells after 0 Gy and 6 Gy radiation in blank, NC, pcDNA3.1-PCAT1 and PCMV-shPCAT1 groups. Figures were compared with NC group and all data were shown as mean ± SD of three experiments (***$P < 0.001$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. NC group). **b** After transfection 24 h, 48 h, 72 h and 96 h, the cell proliferation was examined by CCK-8 assay respectively in blank, NC, pcDNA3.1-PCAT1 and PCMV-shPCAT1 groups (**$P < 0.05$, ***$P < 0.01$ vs. NC group). **c, d** RT-qPCR analysis of relative expression levels of miR-129-5p and HMGB1 in GSCs after transfection with pcDNA3.1-PCAT1 or PCMV-shPCAT1 (***$P < 0.01$ vs. NC group)

after the upregulation of PCAT1, CCK8 assay was performed in our study. We detected the OD value at 24 h, 48 h, 72 h, and 96 h. Compared with the NC group, the cell proliferation was increased in the pcDNA3.1-PCAT1 group (Fig. 4b). PCAT1 functions as competing endogenous RNA (ceRNA) to promote invasion and metastasis via the miR-129-5p-HMGB1 signaling pathway in hepatocellular carcinoma reported by Zhang et al. [4]. To further explore whether PCAT-1/miR-129-5p/HMGB1A (High Mobility Group Box 1) axis play a role in GSCs, we detect the mRNA levels of miR-129-5p and HMGB1 by RT-qPCR assay after altering the expression of PCAT1 in glioma stem cells. The overexpression of miR-129-5p and lower HMGB1 expression were observed in the PCMV-shPCAT1 group, while miR-129-5p was increased and HMGB1 was decreased in the pcDNA3.1-PCAT1 group, which show a hint that the miR-129-5p-HMGB1 axis may be involved in glioma cell carcinoma (Fig. 4c, d).

Discussion

Glioma is reported to be one of the most malignant brain tumors in the central nervous system [1]. Its overall survival rate remained dismal as a result of resistance to radiotherapy and other treatments [5, 21]. It has been found that only a fraction of tumor cells was accountable for glioma regrowth after radiation [9]. To testify this result, the identification of a vital cellular subpopulation of cancer tumor cells (defined as GSCs in glioma) with the characteristics of stem cells, including self-renewal and multipotency capability, provided a penetrating research prospective [6]. In previous studies, GSCs were suggested to enhance resistance to radiation [6, 8] through the regulation of signaling pathways including NOTCH, Wnt/β-catenin, Hedgehog, STAT3, and PI3K/AKT/mTOR [5] or the activation of DNA damage checkpoint response and increase DNA repair capacity [9]. It has been proved that lncRNA PCAT1 played a crucial role in the drug resistance of cells [17] and in the development of glioblastoma [18]. Therefore, we suspected that PCAT1 might also played a role in the radioresistance of GSCs in glioma and explore the underlying associated molecular mechanism.

Radiation therapy, as a major cancer treatment modality, functions based on the theory that cancer cells are more sensitive to radiation compared to normal cells as cancer cells exhibited limited DNA repair capability while normal tissues can recover to withstand radiation [22]. Apoptosis could be induced by irradiation or chemotherapy when DNA was damaged in some cells [23], hence, inadequate apoptotic response was a major indicator of radioresistance [20]. Moreover, γH2AX, a biomarker, has also be defined as an accurate tool to monitor DNA DSBs in cancer researches and a quick read-out of DNA damages [24]. Therefore, we evaluated the response to radiation of GSCs through the measurement of apoptosis rates and percentages of γH2AX positive cells.

Cancer stem cells has been reported to promote radioresistance in various cancer types [6, 8]. Hence, we hypothesized that glioma stem cells might exhibit radiation tolerance in glioma. Expression of cell markers as representative

stem cells should be widely acknowledged, including CD133 and ABCG2, etc. We used CD133 surface marker staining to separate the GSCs and further investigated two specific markers expression in our study. Previous study showed that GSCs had a great capability to form neurospheres in vitro [25]. Similarly, in this study, GSCs were also proved to exhibit sphere-formation capability. Meanwhile, γH2AX positive cells level was examined as a means to measure the ability of cells to damage and repair DNA after irradiation [26]. Experiments showed that stem cells exhibited lower rate of γH2AX positive cells. Furthermore, our study showed that apoptosis rates after radiation treatment of 6 Gy of both glioma cancer cells and GSCs were increased compared with those of 0 Gy group. In addition, stem cells showing a smaller change in apoptosis rate after 6 Gy radiation, which could be concluded as more insensitive to radiation in comparison with cancer cells. Silencing PCAT-1 could inhibit the cell proliferation in prostate cancer [27] and gastric cancer cells [28]. Thus, we also investigated the influence of silencing PCAT-1 on GSCs proliferation. The result showed that knockdown of PCAT-1 inhibited the cell proliferation. Long non-coding RNA PCAT-1 promotes invasion via the miR-129-5p-HMGB1 signaling pathway [4]. According our results, low expression of miR-129-5p and high expression of HMGB1 were observed in pcDNA3.1-PCAT-1 group, which has a hint for us that the miR-129-5p-HMGB1 axis may participate in the progress of the glioma. These evidences suggested that GSCs was relatively insensitive to radiation compared to cancer cells. These findings were in agreement with a string of existing researches [5, 6, 8]. Therefore, it was of great significance to investigate the underlying molecular mechanism so as to investigate the target GSCs as potential therapy.

Mounting evidences have revealed that lncRNAs were ubiquitously dysregulated in tumor cells and have crucial regulatory roles in the malignant progression of tumor cells, such as proliferation, migration and invasion, and apoptosis [12]. In the research field of glioma stem cells, many lncRNAs have been identified to exert their impacts, such as lncRNA HOTAIR [29], lncRNA NEAT1 (Nuclear paraspeckle assembly transcript 1), lncRNA XIST and lincRNA ROR (Reprogramming-Related lncRNA) etc. [12, 30, 31]. However, PCAT1-associated literatures were limited. In this study we targeted PCAT1 as the lncRNA of the interest of this subject, as it was highly upregulated in the GSCs than in cancer cells. This was in agreement with the finding in another experiment which compared the expression profiles of 83 lncRNA genes and discovered that PCAT-1 showed high expression in stem cells than in cancer cells [18]. In HCC and prostate cancer [14], colorectal cancer (CRC) [15], and esophageal squamous cell carcinoma (ESCC) [16], PCAT1 was already found to contribute to poor prognosis. Consistently, in our experiments we also discovered that the upregulation of PCAT1 facilitated radioresistance as it decreased the level of γH2AX positive cells and the proportion of apoptosis cells, while the downregulation exhibited opposite results. This indicated that GSCs promoted glioma progression and radioresistance through the regulation of PCAT1.

The major limitation of this study was a lack of in vivo study. While this cell experiments could closely measure the characteristics of in vivo study, it would be more accurate if an in vivo study was conducted. Moreover, clinical practices were expected to be investigated to testify the outcome of this study. We expected that this would be improved by future researches.

Conclusion

In this study, it was proved that knockdown of PCAT1 in GSCs could reduce sphere formation and increase the level of apoptosis and γH2AX positive cells, indicating that GSCs enhanced radioresistance through the regulation of PCAT1. Therefore, future therapeutic approach could target PCAT1 in GSCs to improve treatment efficacy.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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