Iranian Journal of Basic Medical Sciences
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IJBMS

# Radiosensitizing effects of Sestrin2 in PC3 prostate cancer cells

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**ARTICLE INFO**

***Article type:***
Original article

***Article history:***
Received: Aug 12, 2016
Accepted: Sep 28, 2017

***Keywords:***
Cancer
Prostate
Radiation
Sensitization
Sestrin2

**ABSTRACT**

***Objective(s)***: The stress-responsive genes of Sestrin family are recognized as new tumor suppressor genes in breast carcinoma, however, the function of Sestrin family in human prostate cancer is not clear. Ionizing radiation (IR) is known to induce Sestrin gene expression in breast cancer cells. However, the response of Sestrin to IR has not been reported in PC3 prostate cancer cells.

***Materials and Methods:*** Sestrin2 expression in prostate cancer cell lines (PC3, LNCaP clone FGC, and DU145) was detected by Western blot and real-time PCR. Cell counting kit (CCK-8) was used to detect cellular proliferation. The radiosensitivity of PC3 cells was detected by clonogenic assay.

***Results:*** Sestrin2 expression in prostate cancer cell lines (PC3, LNCaP clone FGC, and DU145) is low. *In vitro* assays indicated that over-expressing Sestrin2 in human prostate cancer PC3 inhibited tumor proliferation. In addition, elevated Sestrin2 expression sensitized PC3 cells to IR.

***Conclusion:*** We determined Sestrin2 may function as a tumor suppressor through repressing proliferation, mediating sensitization to IR in PC3 cells.

▶ *Please cite this article as:*
Fu H, Song W, Wang Y, Deng W, Tang T, Fan W, Qu Sh. Radiosensitizing effects of Sestrin2 in PC3 prostate cancer cells. Iran J Basic Med Sci 2018; 21:621-624. doi: 10.22038/IJBMS.2018.18283.4923

## Introduction

In the developed countries the most common cancer in men is prostate cancer, while the incidence of prostate cancer is low in China. The rate of age-standardized incidence of prostate cancer is much lower in China compared with the rates in American and European countries, based on the global estimates data (1, 2). However, the incidence rate of prostate cancer had a remarkable increase from 2000 to 2009 in city of Shanghai due to the changes of lifestyle and dietary habits (3). It has been shown that hormonal and surgical therapies are helpful for hormone-responsive, early-stage disease. However, most patients with prostate cancer are diagnosed in the last stages and few treatment options are available at this stage. The progression of prostate cancer is due to imbalance of tumor suppressor genes and tumor-promoting genes. So, gene therapy is a new effective treatment option for drug-resistant and refractory prostate cancer (4, 5).

Sestrins are known as a stress-inducible proteins family that are conserved in many species including *Drosophila melanogaster*, *Caenorhabditis elegans*, and mammals, and regulate metabolic homeostasis (6, 7). The Sestrin family includes 3 different members (Sestrin1-3) in mammals. Sestrin2 has been widely studied in the adipose and liver tissue (6, 8, 9). Sestrin2 plays an important role in the regulation of growth arrest and DNA damage under different cellular pressures (10).

Radiation therapy is exposure to ionizing radiation (IR). It induces DNA damage, which in turn facilitates cell cycle arrest through activation of the kinase ataxia telangiectasia mutated (ATM) (11). IR is known to up-regulate Sestrin2 expression in breast cancer cells (12, 13) and repair DNA damage or promote apoptosis.

In this study, we detected Sestrin2 expression in prostate cancer cell lines, including PC3, LNCaP clone FGC, and DU145. In addition, we observed the role of Sestrin2 in radiosensitization of prostate cancer cells.

## Materials and Methods

### *Materials*

RPMI medium, DMEM medium, and fetal bovine serum (FBS) were from Invitrogen. Antibodies against GAPDH and Sestrin2 were from Cell Signaling Technology. Human prostate epithelial cell RWPE-1, LNCaP clone FGC, DU145, and PC3 were from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China).

### *Cell culture and treatments*

RWPE-1 was incubated in K-SMF medium supplemented with EGF (5 ng/ml) and bovine pituitary extract (50 μg/ml). LNCaP clone FGC and PC3 were incubated in RPMI 1640 medium, DU145 in DMEM; all media supplemented with FBS (10%), streptomycin (100 μg/ml), and penicillin(100 U/ml) at 37 ˚C with 5% $CO_2$. 2 to 8 Gy IR was used to treat PC3 cells with a clinical linear accelerator radiotherapy unit. PC3 cells were transfected with pCMV-Vector or pCMV-Sestrin2

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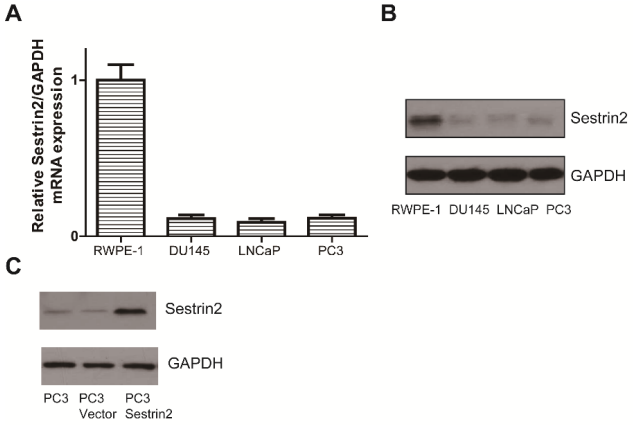


**Figure 1.** Low Sestrin2 expression in prostate cancer cell lines. A. mRNA expression of Sestrin2 was much lower in LNCaP clone FGC, DU145, and PC3 than that in RWPE-1 cells. B. Sestrin2 protein expression was much lower in LNCaP clone FGC, DU145, and PC3 than that in RWPE-1 cells. C. PC3 cells were transfected with pCMV-Sestrin2 or pCMV-Vector plasmid, expression of Sestrin2 was significantly higher than in PC3-Vector cell line

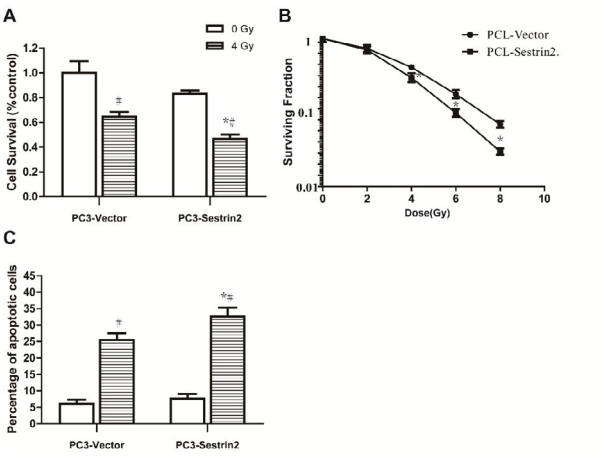


**Figure 2.** IR combination with Sestrin2 overexpression modulates cell survival and increases cell radiosensitization. A. Clonogenic efficiency of PC3 cells pretreated with pCMV-Sestrin2 or pCMV-Vector plasmids, then exposed to 4 Gy or 0 Gy of IR. 48 hr later PC3 cell proliferation was determined by CCK-8 cell assay. B. PC3-Sestrin2 cells or PC3-Vector cells were exposed to different doses (0, 2, 4, 6, and 8 Gy) of IR. After 3 days, PC3 cells were fixed with formaldehyde and then methylene blue was used to stain and the clonogenic survival was counted. C. PC3-Sestrin2 cells or PC3-Vector cells were exposed to 4 Gy or 0 Gy of IR. PE-Annexin V staining was used to detect cell apoptosis by FACS analysis 48 hr later. Data shown is expressed as the mean±SEM of at least three independent experiments. #$P$<0.05 vs 0 Gy group; *$P$<0.05 vs Vector group

plasmids by Lipofectamine 2000 (14).

### *Real-time PCR*

Total RNA extraction was from PC3 cells, and cDNA fragments and real-time PCR was performed as before (15). The human Sestrin2 primer sequences were: forward 5'-GCGAGATCAACAAG TTGCTGG-3', reverse 5'-ACAGCCAAACACGAAGGAGG-3'.

### *Cell proliferation assay*

After being pretreated with PC3-Sestrin2 or PC3-Vector, cells were treated with a single dose of 0 Gy or 4 Gy IR. After irradiation, cells were reseeded into 96-well flat plates to incubate at 37 ˚C for 2 days, cell counting kit (CCK-8) was used to detect cellular proliferation by manufacturer's instructions. The absorbance was studied at 450 nm wavelength and adjusted at 690 nm wavelength.

### *Clonogenic assay*

The radiosensitivity of PC3 cells was detected by clonogenic assay (12). After being pretreated with PC3-Vector or PC3-Sestrin2, cells were treated with a single dose of 0–8 Gy IR. After irradiation, cells were reseeded in culture 100 mm dishes immediately, to incubate at 37 ˚C for 7 days to allow colony formation. At the end of the incubation, methylene blue was used to fix cells and viable colonies were calculated. GraphPad Prism 5 software was used to assess radiation sensitization by Sestrin2.

### *Western blot analysis*

SDS-PAGE was used to separate protein, and PVDF membranes were used to transfer; electrophoresis and immunoblotting were employed according to a previous publication (15).

### *Apoptosis detection by FACS*

At 48 hr post-irradiation, PC3 cells were collected. After incubation with PE-Annexin-V(BD Biosciences), cells were resuspended in PBS solutions containing 2% paraformaldehyde and indicated by FACS. The percentage of apoptosis cells was determined as before (15).

### *Statistical analysis*

The results are all expressed as mean±SEM. Significant differences ($P$<0.05) were found between the two groups by student's t-test.

## Results

### *Low Sestrin2 expression in prostate cancer cell lines*

Sestrin2 mRNA and protein expression in LNCaP clone FGC, DU145, and PC3 cell lines was significantly lower than that in the RWPE-1 cell line (normal prostate epithelial) (Figures 1A, B).

### *Overexpression of Sestrin2 decelerates PC3 cell proliferation and increases radiosensitization*

To study the Sestrin2 gene's potential role of inhibiting cell growth, PC3 cells were transfected with pCMV-Sestrin2 or pCMV-Vector plasmid, Sestrin2 expression in pCMV-Sestrin2 cell line was significantly higher compared with Sestrin2 expression in the PC3-Vector cell line by Western blot (Figure 1C). As shown in Figure 2, after cell seeding for 48 hr, the deceleration of cell growth in PC3-Sestrin2 cell line was observed compared with PC3-Vector cell line ($P$<0.05). To study the possible effects of Sestrin2 on IR treatment, a clonogenic survival assay using IR was conducted (Figure 2B). As we expected, IR alone treatment decreased clonogenic survival in PC3 cells in a dose-dependent manner. At the same time, overexpression of Sestrin2 had significant radiosensitizing combination effects with 4Gy IR (Figure 2B). In addition, Sestrin2 overexpression significantly increased PC3 cell apoptosis after IR treatment (Figure 2C).

## Discussion

Sestrins family is conserved throughout all kinds of species. *In vitro* studies indicated that Sestrins have an antioxidant function that suppresses reactive oxygen species (ROS) through restoration of overoxidized peroxiredoxins (7). Recently, Sestrins have been shown to be involved in some physiological processes, which are independent of their redox state (16). Sestrins are negative feedback regulators, which target rapamycin (TOR) via AMPK regulation in *D. melanogaster,* and Sestrins depletion leads to age-associated obesity-induced pathologies, such as protein aggregate formation, lipid accumulation, mitochondrial dysfunction, and muscle degeneration (17). However, mammalian Sestrin1/2 was indicated to form an active complex with TSC2 affecting mammalian-TOR (mTOR) and AMPK signal pathway during genotoxic stress (17). Furthermore, it has also been confirmed that Sestrin2 plays a key role in regulating autophagy and inhibiting tumor proliferation (12, 18, 19).

Recent data indicates Sestrin2 plays a key role in many kinds of cancer cell lines. Sestrin2 activates the AKT pathway and promotes cell survival in response to chemotherapeutics and UVB radiation stress, which suggests that Sestrin2 is oncogenic in skin melanoma and SCC (20). Sestrin2-mediated regulation of mTORC1 and mTORC2 is necessary for the survival of glutamine-depleted lung cancer cells (21). Sanli *et al.* (12) suggested AMPK pathway is involved in basal and IR-induced Sestrin2 expression regulation in cancer cells, which regulates IR sensitization of breast cancer cells. Our results indicated that it has a much lower Sestrin2 expression in DU145, PC3, and LNCaP clone FGC prostate cancer cell lines compared with RWPE-1 (normal prostate epithelial cell line). PC3 cell line has been widely used in progressive stages of prostate cancer cell models, and it is more similar to some castration-resistant prostate cancers in clinical conditions. It was chosen in our study as a progressive stage of prostate cancer model to explore the role of Sestrin2 in cancer sensitization and proliferation to IR (22, 23). To study the Sestrin2 gene's role in PC3 cell growth, cells were transfected with pCMV-Sestrin2 or pCMV-Vector plasmid; Sestrin2 expression in PC3-Sestrin2 cells was much higher compared with the PC3-Vector cells. Our data suggested that exogenous over-expression of Sestrin2 decelerates PC3 cell proliferation.

To explore the effects of overexpression of Sestrin2 on sensitization to IR treatment, a clonogenic survival assay using IR was conducted. It has been previously reported that overexpression of Sestrin2 modulated cell viability under stress (9).As we expected, IR treatment decreased PC3 cells clonogenic survival in a dose-dependent manner, and this effect was significantly enhanced when combining IR (4 Gy) with Sestrin2 overexpression. In addition, overexpression of Sestrin2 significantly increased PC3 cell apoptosis after IR treatment. The detailed mechanism of cell apoptosis should be explored in future experiments.

## Conclusion

Briefly, our data indicates a novel role of Sestrin2 in ionizing irradiation in prostate cancer cells. Sestrin2 inhibits cell survival after IR stress, we show for the first time that Sestrin2 increases sensitivity under radiation in prostate cancer cells. In summary, our data suggests that Sestrin2 is a stress-sensitive gene. In addition, our research supported that Sestrin2 may act as a tumor suppressor by mediating sensitization, repressing proliferation to IR in PC3 cells. It may provide a new molecular signal pathway network through Sestrin2 upregulation and offer the key oncogenic role of Sestrin2 in prostate cancer cells. Future research should explore the specific signal pathway which up-regulates Sestrin2 expression and study Sestrin2's potential role to act as a transcription factor, which regulates stress-related gene expression.

## Acknowledgment

This work was supported by grants from the National Natural Science Foundation of China (81670424), the Key Scientific Research Fund of Hunan Provincial Education Department (15A166), the Scientific Research Fund of Hunan Provincial Health and Family Planning Commission(B2016087), the National Natural Science Foundation of Hunan Province (2015JJ2118), the Aid Program for Science and Technology Innovative Research Team in Higher Educational Institutions of Hunan Province (2008-244), the Construct Program of the Key Discipline in Hunan Province (2011-76), and Postgraduate Innovation Project of Scientific Research of University of South China (2015XCX38).

## Conflicts of Interest

The authors have no conflicts of interest to declare.

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