

Original Research Article

Deltex E3 Ubiquitin Ligase 3L confers radioresistance in prostate cancer via Akt pathway

Junyi Xiang¹, Bodong Lv², Shufeng Fan¹, Zhitian Zhang¹, Hui Yang^{3*}

¹Department of Medical Imaging, ²Department of Urology Surgery, The Second Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou City, Zhejiang Province 310000, ³Department of Urology Surgery, Qianjiang Central Hospital of Chongqing, Chongqing City 409000, China

*For correspondence: **Email:** HUYOKL87yh@163.com; **Tel:** +86-23-979245641

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Abstract

Purpose: To determine the effect of Deltex E3 Ubiquitin Ligase 3L (DTX3L) on the radioresistance of prostate cancer (PCa).

Methods: A PCa cell model of radioresistance was established via exposure of cancer cell lines to fractionated radiation. The MTT {(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)} assay and western blotting were performed to evaluate the impact of DTX3L on cell survival and DNA damage repair. The molecular mechanism of action was evaluated by western blotting.

Results: DTX3L was elevated in PCa cell lines compared with normal primary prostate epithelial cells ($p < 0.01$). The survival of PCa cells exposed to radiation was promoted by overexpression of DTX3L, while knockdown of DTX3L abrogated the radioresistance. Moreover, overexpression of DTX3L decreased phosphorylation of histone H2AX (γ H2AX) and increased Rad51 levels ($p < 0.01$). However, knockdown of DTX3L reversed the accumulation of γ H2AX and Rad51. Phosphorylation of AKT was promoted by DTX3L overexpression, but was reduced by DTX3L knockdown ($p < 0.01$). Inhibition of AKT (protein kinase B) counteracted the promotion ability of DTX3L on the radioresistance of PCa cells via decreased cell survival ratio, and also inhibited DNA damage repair via accumulation of γ -H2AX and depletion of Rad51 ($p < 0.01$).

Conclusion: DTX3L increases the resistance of prostate cancer to radiotherapy and DNA damage repair in PCa via AKT pathway, indicating a potential therapeutic strategy to overcome radioresistance in PCa.

Keywords: DTX3L (Deltex E3 Ubiquitin Ligase 3L), DNA damage, Phosphorylation, Radioresistance, AKT, Protein kinase B, Prostate cancer

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INTRODUCTION

Prostate cancer (PCa) is common in males, with an increasing number of cases being diagnosed in the past decade [1]. Traditional treatments, including surgical resection, endocrine therapy,

chemotherapy, and radiation therapy (RT) are effective for PCa [2]. RT is one of the most commonly used therapies for localized PCa due to its noninvasive nature and low risk [2]. However, the recurrence of PCa accompanied with radioresistance of PCa cells makes PCa a

fatal disease with a high mortality rate [2]. Therefore, new therapeutic targets are needed to identify and improve the radiosensitivity of PCa.

DTX3L (Deltex E3 Ubiquitin Ligase 3L) is an E3 ligase, which is widely expressed in different tissues with higher expression in the thymus [3]. DTX3L can bind with B invasive lymphoma 1, which is important for DLCLB (diffuse large B-cell lymphoma) [4]. Previous studies have shown that DTX3L participates in tumor progression, including those in glioma [5] and PCa [6]. DTX3L inhibits the expression of interferon response factor-1 and regulates proliferation of PCa cells [6]. However, the functional role of DTX3L in radioresistance of PCa is still not fully known.

AKT (protein kinase B) has been implicated in the mediation of pathways involved in cell proliferation, survival, and metabolism [7]. Activation of AKT via its regulator, phosphoinositide 3-kinase (PI3K), has shown oncogenic ability in various tumors, such as the promotion of metastasis in PCa [7]. Inhibition of AKT has been widely used for the treatment of PCa [8], and PI3K/AKT activation could result in the development of resistance to chemotherapy [9] and radiotherapy [10]. In addition, DTX3L can regulate the PI3K/AKT pathway to promote metastasis of melanoma [11]. Therefore, AKT might be associated with DTX3L-mediated radioresistance in PCa.

Therefore, this study was conducted to determine the relationship between DTX3L and the radioresistance in PCa cells. Furthermore, the effect of DTX3L on the AKT pathway involved in the radioresistance of PCa was also evaluated. The results could facilitate the development of a potent therapeutic target to overcome radioresistance of PCa.

EXPERIMENTAL

Cell culture

PCa cell lines (PC3, DU145, and LNCaP) and HPE were cultured in RPMI 1640 medium (Life Technologies, Gaithersburg, MD, USA) with 10 % fetal bovine serum (Gibco, Grand Island, NY, USA) at 37 °C and 5 % CO₂.

Cell transfection and treatment

Full-length DTX3L DNA was inserted into the vector pcDNA 4.1 (Invitrogen, Carlsbad, CA, USA). To silence DTX3L, two short hairpin RNAs (shRNAs; shDTX3L 1# and 2#) were synthesized by GenePharma (Suzhou, China). PC3 cells were transfected with pcDNA-DTX3L or the

negative control (vector), and DU145 cells were transfected with shRNAs (20 nM) or the negative control (shNC) using Lipofectamine 2000 (Invitrogen).

The cells, after different transfections, were treated with or without 2.5 μM MK-2206 for 24 h. The cells were then treated with 0, 2, 4, 6, or 8 Gy ionizing radiation using a ⁶⁰Co clinical unit for 1 h before the functional assays.

Cell viability

PC3 or DU145 cells (9 × 10⁴ cells/well) after ionizing radiation treatment were seeded and incubated for 24 h. Each well was incubated with 10 μL MTT solution (Dojindo, Tokyo, Japan) for 4 h, and then incubated with 100 μL dimethyl sulfoxide. Absorbance at 570 nm for each well was determined using a micro ELISA reader (Bio-Tek, Winooski, VT, USA).

Western blotting

Proteins were extracted from PC3 or DU145 cells and subjected to SDS-PAGE. The proteins were transferred to a polyvinylidene fluoride membrane, and the membrane was incubated with the following primary antibodies: anti-DTX3L (1:1,500; Abcam, Cambridge, UK); γ-H2AX and Rad51 (1:2,000, Abcam); Akt and p-Akt (1:2,500, Abcam); and β-actin (1:3,000, Abcam) after blocking in 5% bovine serum albumin. The membrane was then incubated with horseradish peroxidase-labeled secondary antibody (Sungene, Tianjin, China), and the immunoreactivities were determined using the Millipore ECL system (Millipore, Billerica, MA, USA).

Statistical analysis

Data are shown as the mean ± standard error of mean and were analyzed using Prism 6 software (GraphPad, San Diego, CA, USA). Statistical analyses were conducted using one-way analysis of variance. A value of **p* < 0.05 or ***p* < 0.01 was considered statistically significant.

RESULTS

DTX3L conferred radioresistance to PCa cells

Western blotting showed higher expression of DTX3L in PCa cells (PC3, DU145, and LNCaP) than in HPE cells (Figure 1 A). PC3 cells with the lowest expression of DTX3L were transfected with pcDNA-DTX3L (Figure 1 B), and DU145 cells with the highest DTX3L expression were transfected with shRNAs targeting DTX3L

(Figure 1 B). Survival curves of PC3 or DU145 cells after radiation were then evaluated. The results indicated that PC3 cells overexpressing DTX3L showed higher survival than the negative control (Figure 1 C). However, DU145 cells with silenced DTX3L showed lower survival than the negative control (Figure 1 C), demonstrating that DTX3L conferred radioresistance in PCa cells.

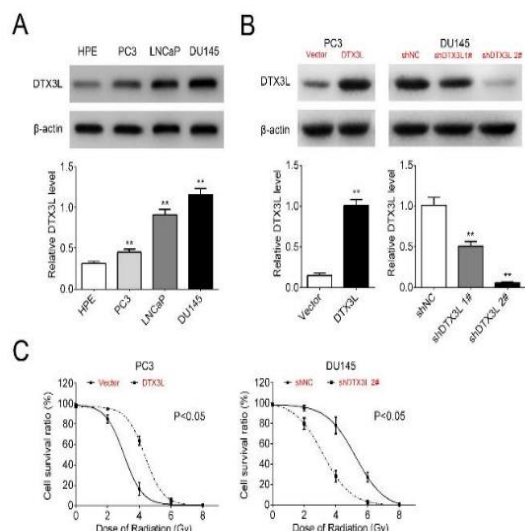


Figure 1: DTX3L conferred radioresistance in PCa cells. (A) Protein expression of DTX3L in PCa cells (PC3, DU145, LNCaP) and HPE. (B) Transfection of pcDNA-DTX3L or shRNAs targeting DTX3L (shDTX3L 1#, 2#) in PC3 or DU145 cells detected by western blotting. (C) The effect of DTX3L on the viability of PC3 or DU145 cells after treatment with 0, 2, 4, 6, or 8 Gy radiation, detected using the MTT assay; ** $p < 0.01$

DTX3L promoted DNA damage repair in radioresistant PCa cells

Overexpression of DTX3L resulted in decreased γ H2AX and increased Rad51 levels (Figure 2 A), thus promoting DNA damage repair to enhance survival in radioresistant PCa cells. However, knockdown of DTX3L increased the protein expression levels of γ H2AX, while it decreased Rad51 expression to enhance DNA damage in radioresistant PCa cells (Figure 2 B).

DTX3L promoted AKT activation in radioresistant PCa cells

Data from western blotting showed that DTX3L increased phosphorylation of AKT in PC3 cells after treatment with 4 Gy radiation (Figure 3 A), while knockdown of DTX3L decreased phosphorylation of AKT in DU145 cells (Figure 3 B), suggesting that DTX3L promoted AKT activation in radioresistant PCa cells.

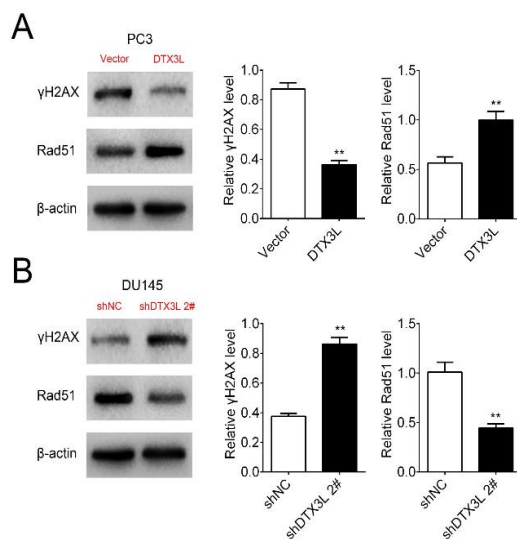


Figure 2: DTX3L promoted DNA damage repair in radioresistant PCa cells. (A) The effect of DTX3L on protein expression levels of γ H2AX and Rad51. (B) The effect of shDTX3L 2# on protein expression levels of γ H2AX and Rad51; ** $p < 0.01$

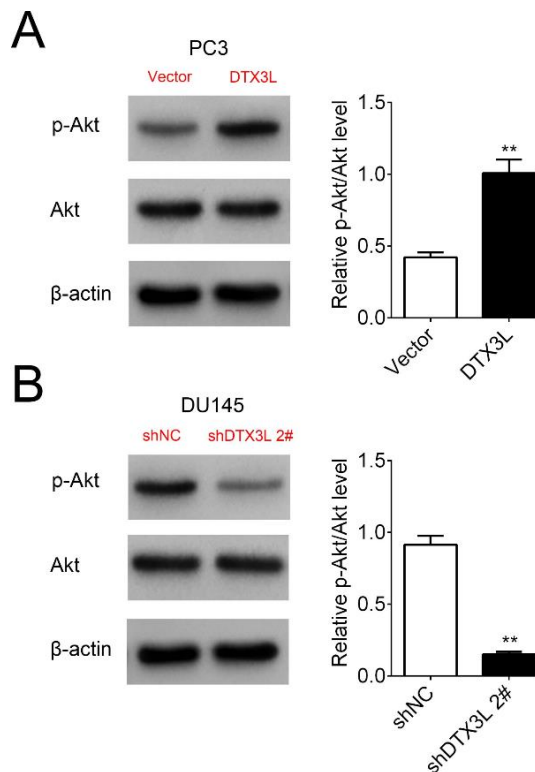


Figure 3: DTX3L promoted AKT activation in radioresistant PCa cells. (A) The effect of DTX3L on protein expression of p-Akt and Akt. (B) The effect of shDTX3L 2# on protein expression levels of p-Akt and Akt; ** $p < 0.01$

Inhibition of AKT attenuated DTX3L-induced radioresistance in PCa cells.

Inhibition of AKT via MK-2206 treatment decreased the viability of radioresistant PC3 cells (Figure 4 A), while overexpression of DTX3L increased the viability of radioresistant PC3 cells after MK-2206 treatment (Figure 4 A). Moreover, DNA damage repair was suppressed by MK-2206 treatment in radioresistant PC3 cells with increased γ H2AX and decreased Rad51 levels (Figure 4 B and C). However, overexpression of DTX3L attenuated the MK-2206-induced increase of γ H2AX and decrease of Rad51 levels (Figure 4 B and C). Furthermore, DTX3L overexpression reversed the suppressive effect of MK-2206 on AKT phosphorylation in radioresistant PC3 cells (Figure 4 B and C), suggesting that the inhibition of AKT attenuated DTX3L-induced radioresistance in PCa cells.

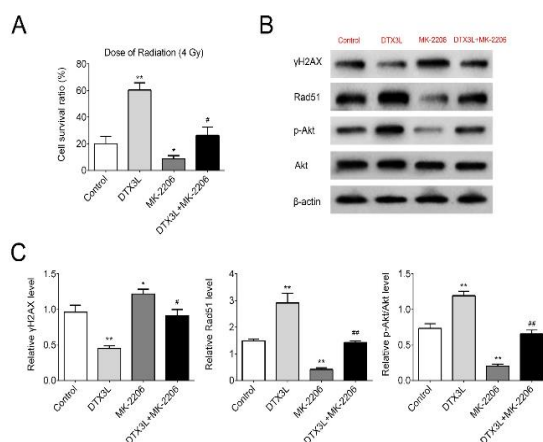


Figure 4: Inhibition of AKT attenuated DTX3L-induced radioresistance in PCa cells. (A) The effect of DTX3L and MK-2206 on the viability of PC3 cells after radiation. (B) The effect of DTX3L and MK-2206 on protein expression levels of γ H2AX, Rad51, p-Akt, and Akt. (C) The relative γ H2AX, Rad51 p-Akt, and Akt levels affected by DTX3L and MK-2206. *DTX3L vs. control or MK-2206 vs. DTX3L; $p < 0.05$, $p < 0.01$; #, ##DTX3L + MK-2206 vs. MK-2206, $p < 0.05$, $p < 0.01$

DISCUSSION

Previous research has shown that inhibition of DTX3L suppressed chemoresistance in PCa cells [6]. The present study was then conducted to evaluate whether inhibition of DTX3L increased the efficiency of radiation treatment in PCa.

The results showed that DTX3L was elevated in PCa cells [12] and chemotherapy-resistant DLCBL [13]. Knockdown of DTX3L inhibited survival of chemoresistant PCa cells [6]. The

results showed that DTX3L overexpression increased the cell survival ratio in radioresistant PCa cells, while knockdown of DTX3L reduced cell viability. Therefore, DTX3L might have decreased the efficiency of radiation treatment in PCa cells.

Evidence has shown that regulation of DNA damage responses could confer radioresistance to tumor cells [14]. DTX3L was shown to mono-ubiquitinate histone H4, thereby modulating the DNA damage response [15]. Moreover, DTX3L may be recruited to radiation sites for the progression of DNA damage repair, providing resistance to DNA-damaging chemotherapeutic agents [16]. Inhibition of DTX3L increased the efficiency of chemotherapeutic agents or radiation treatment [15]. The present study showed that DTX3L promoted DNA damage repair in radioresistant PCa cells with decreased γ H2AX and increased Rad51 levels. Previous studies showed that DTX3L promoted DNA repair via nonhomologous end-joining [17]. However, Rad51 functions as a critical regulator of homologous recombination through binding with single-stranded DNA, thereby repressing access for nonhomologous end-joining [18]. However, the detailed effect of DTX3L on DNA damage repair pathways needs to be investigated in further studies.

Previous research has shown that hyper-activation of the PI3K/AKT pathway is implicated in the radioresistance of PCa [19]. Activation of AKT could increase phosphorylation of downstream targets involved in cell survival, cell cycle, cell apoptosis, and tumor heterogeneity to promote the development of radioresistance in PCa [20]. Moreover, epithelial plasticity, including the epithelial-mesenchymal transition, has also been regarded as a critical driver of radioresistance in PCa [20].

Data from the present study also confirmed that DTX3L promoted the activation of the AKT pathway via phosphorylation of Akt. However, the functional roles of DTX3L on the cell cycle, apoptosis, and the epithelial-mesenchymal transition in radioresistant PCa cells remain elusive. Furthermore, previous studies showed that DTX3L functioned as an activator of signal transduction and transcription signaling (STAT) in the regulation of chemoresistance and survival in PCa cells [6]. In addition, activation of STAT resulted in the dimerization of STAT and its translocation to the nucleus to bind with the promoter region of Akt [21]. Therefore, DTX3L might confer radioresistance in PCa via AKT-STAT pathway.

Inhibitors of the AKT pathway could be effective for increasing radiosensitivity of PCa [22]. For example, palomid 529 increased radiosensitivity in PC-3 cells in combination with radiation therapy [23]. Arsenic trioxide, an Akt inhibitor, could enhance radiosensitivity in LNCaP and PC-3 cells [24]. In the present study, consistent with the results of Thang *et al* [11], knockdown of DTX3L decreased phosphorylation of Akt. In addition, inhibition of AKT via MK-2206 attenuated DTX3L-induced radioresistance in PCa cells. Furthermore, various resistance pathways, including adaptive, DNA damage repair, inflammation, and hypoxic pathways are involved in the resistance against radiotherapy [25]. However, whether DTX3L regulates other resistance pathways during radioresistance in PCa needs to be investigated.

CONCLUSION

The findings of this study show that DTX3L confers radioresistance on PCa cells via regulation of Akt pathway. Thus, DTX3L might, therefore, be a potential therapeutic target for the improvement of radioresistance in PCa.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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