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ORIGINAL RESEARCH

Microtubule-associated protein tau is associated with the resistance to docetaxel in prostate cancer cell lines

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Abstract: Tau, a microtubule-associated protein, has been investigated primarily in neurons. Recently, tau has been explored to be associated with increased drug resistance in various kinds of cancers. We found that the tau was expressed in prostate cancer cell lines DU145 and PC-3. We also reported that recurrent prostate cancer cells after docetaxel treatment have higher levels of microtubule-associated protein tau. In vitro, inactivation of tau by gene knockdown suppressed cell proliferation and sensitized docetaxel cytotoxicity. Also, our results demonstrated that the PI3K/Akt/mTOR pathway was upregulated in DU145 docetaxel-resistant cells compared with the DU145-naïve cells. Thus, targeting tau protein and PI3K/Akt/mTOR pathway are promising strategies to enhance docetaxel response for the treatment of prostate cancer.

Keywords: prostate cancer, microtubule-associated protein tau, docetaxel resistance, tau protein, PI3K/Akt/mTOR pathway

Introduction

Prostate cancer is still the second leading cause of death among patients with different male malignant tumors. Although majority of the patients respond well to the dominating treatment with prostatectomy followed by docetaxel, however, many of them relapse within a few years after first-line treatment.[1,2]

Resistance to chemotherapeutic drugs remains a challenging and pervasive problem in cancer treatment, and it will be of great significance in clinical medicine if effective strategies to overcome drug resistance are made up.[3] Due to the resistance to cytotoxic chemotherapy, the primary tumors can develop into recurrent tumors. The mechanisms underlying cell resistance to cytotoxic chemotherapeutics that are designed to be selective for particular molecular targets share many same characteristics, such as uncertain alterations of the drug targets, abnormal activation of survival pathways, and ineffectiveness of cell death.[3] Therefore, it is crucial to study recurrent cancer cells and expound the molecular etiology of resistance to chemotherapeutic drugs.

Microtubules are intrinsically dynamic polymers which are composed of a β-tubulin heterodimers. Microtubules dynamically control cellular motility and mitosis by constituting essential components of the mitotic spindle and cytoskeleton. Moreover, microtubules serve as scaffolds for signaling molecules that regulate gene transcription and cell cycle activity.[4]

Microtubule-associated protein tau (MAPT) (50–64 kDa), a product of gene located in chromosome 17 (17q21) which can combine to β-tubulin, is originally purified from neurons. In the previous studies, tau can interact with Fyn and with Pin1 and both the interactions have been thought to be associated with Alzheimer's disease.[5] Moreover,

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direct interaction between Aβ42 and GSK3α facilitates hyperphosphorylation of tau and this mechanism may be a new molecular signaling link in Alzheimer's disease.[6] What's more, in prostate cancer, overexpression of tau was markedly associated with lower Gleason score, indicating that tau may be a potential prognostic marker in prostate cancer.[7] Recently, MAPT has been identified as a promising marker of response to docetaxel in prostate cancer.[8] It may compete with this drug by binding to the interior as well as to the exterior microtubules, which is in the same binding site as docetaxel.[9]

Nevertheless, the mechanisms through which tau mediate docetaxel resistance remain unknown. Notably, the PI3K/Akt/mTOR pathway plays an essential role in the regulation of cell growth, division, and tumorigenesis.[10] Especially, the PI3K/Akt/mTOR pathway is associated with resistance to many kinds of chemotherapeutic drugs. Inactivating this pathway rendered prostate cancer cells more sensitive to drugs targeting microtubules such as paclitaxel, vinblastine, and docetaxel.[11] By investigating PI3K/Akt/mTOR pathway on docetaxel-resistant (DR) prostate cancer cell lines and its parental cells, we attempted to identify whether this signaling pathway is associated with docetaxel resistance. Furthermore, we attempted to explore the relationship between PI3K/Akt/mTOR pathway and tau protein in the docetaxel resistance of prostate cancer.

To explore the MAPT in resistance of docetaxel in prostate cancer, we previously studied three prostate cancer cell lines, LNCaP, PC-3, and DU145, from which DR clones were previously established for our experiment.

The purpose of this study is to find out how and the degree to which tau contributes to resistance to chemotherapeutic drugs in prostate cancers and to put forward a molecular biological foundation for inactivation of tau to strengthen the antitumor effects of chemotherapeutic drugs. Also, we attempted to clarify that MAPT is associated with the resistance to docetaxel in prostate cancer cell lines by regulating the PI3K/Akt/mTOR signaling pathway probably. The results have universal revelation to promote the effectiveness of chemotherapy and improve clinical outcomes in patients with prostate cancer.

Materials and methods

Cell culture and resistant cell line development

The human prostate cancer cell lines that were purchased from the American Type Culture Collection including LNCaP, PC-3, and DU145 were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 50 U/mL penicillin (Sigma-Aldrich, St Louis, MO, USA), 50 μg/mL streptomycin (Sigma-Aldrich), and 2 mM L-glutamine (Invitrogen, Carlsbad, CA, USA).

PC-3-resistant sublines (PC-3 DR) were generated by treating with docetaxel (Sigma-Aldrich) at 4 and 8 nM (suspended in dimethyl sulfoxide) in 75 cm^2 flasks for 48 h. After treatment, the surviving cells were re-seeded into new flasks and allowed to recover for 2–3 weeks. The dose of docetaxel was increased from 4 and 8 nM to 8 and 12 nM, respectively, after 7 (at 4 nM) and 5 (at 8 nM) treatments. In total, the cells underwent 18 treatment cycles at 8 nM and 12 treatment cycles at 12 nM. After each treatment, they were allowed to adequately recover before evaluating the resistance to docetaxel and other experimental work. As the passage number of these treated cells increased over time, a subset of PC-3 cells was aged alongside these cells as a control to ensure that the effects observed were due to resistance rather than the aging effect.[1]

DU145-resistant sublines (DU145 DR) were generated over a period of 6 months by progressively increased dose of docetaxel. Cells were continuously maintained in docetaxel, with treatments beginning at the initial IC_{50} of the respective parent cell lines. Media containing docetaxel were changed every 2–3 days. As cells displayed resistance to docetaxel, the concentration was subsequently increased to the final dose of 100 nM. Resistance was judged by the increased proliferation of cells and the decreased cell death.[1]

Total cellular protein isolation and western blotting assay

Whole cell lysates were extracted from treated cells grown to 90% confluence on T75 flasks and six-well plates. Equal amounts of cell protein lysates (50 μg) were separated by 10% sodium dodecylsulfate–polyacrylamide gel electrophoresis before being transferred onto NC membranes (Sigma-Aldrich) and incubated with specific antibodies to tau (1/1000; Thermo Fisher Scientific, Waltham, MA, USA), p-Akt (1/1000; Abcam, Cambridge, MA, USA), p-mTOR (1/1000; Cell Signaling Technology, Danvers, MA, USA), p-4E-BP1 (1/1000; Cell Signaling), and p-S-6K (1/1000; Abcam), and GAPDH antibody was used as the control.

Cell proliferation assay

Cell viability was assessed by using a Cell-Counting Kit 8 (CCK-8), as described by the manufacturer (Dojindo, Kumamoto, Japan). Twenty-four hours after seeding cells (2×10^3 cells/well) in 96-well plates, cells were transfected with plasmids and then cultured for 1, 2, 3, 4, 5, and 6 days.

The medium in each well was replaced with 100 μL of complete medium containing 10 μL of CCK-8 solution, and the plate was incubated for 1 h at 37°C. The absorbance value was determined at 450 nm using the Infinite M200 spectrophotometer (Tecan, Germany), and each experiment was repeated three times. IC_{50} values were calculated by the GraphPad Prism software.

shRNA lentivirus-mediated knockdown of tau

The shTau-1 target sequence was 5′-GACGCTG-GCCTGAAAGAATCT-3′, the shTau-2 was 5′-GATGC-TAAGAGCACTCCAACA-3′, and the shTau-3 was 5′-GATTTCCTCTCCAAAGTTTCC-3′, and these were cloned in pLKO.1 (Genechem, Shanghai, People's Republic of China). Scramble control (5′-CCTAAGGTTAAGTC-GCCCTCG-3′) was also purchased from Genechem. Retroviral and lentiviral particles were produced by transfecting prostate cancer cells with the appropriate expression and packaging plasmids using Lentiviral Packaging Systems (Genechem) and filtering cultured supernatants through a 0.45-μM filter.

RNA extraction and qRT-PCR analyses

Total RNA was extracted from cultured cells using Trizol reagent (Invitrogen). For qRT-PCR, RNA was reversely transcribed to cDNA by using a Reverse Transcription Kit (TaKaRa Biotechnology, Dalian, People's Republic of China). Real-time PCR analyses were performed using the SYBR Green (TaKaRa Biotechnology) to examine the expression level of tau. Results were normalized to the expression level of 18S rRNA. The assay was conducted using the ABI 7300 system (Applied Biosystems). Specific PCR primers for the tau mRNAs were purchased from Guangzhou RiboBio Co. Ltd. All reactions were done in triplicate, and the expression level of tau was calculated according to the equation $2^{-\Delta\Delta C_t}$. The sequences of primers were as follows: MAPT, forward primer: 5′-TAG GCA ACA TCC ATCATA AAC CA-3′, reverse primer: 5′-TCG ACT GGACTC TGT CCT TGA A-3[12]; 18S rRNA, forward primer: 5′-ACACGGACAGGATTGACAGA-3′, reverse primer: 5′-GGACATCTAAGGGCATCACA-3′.

Statistical analysis

Statistical significance of differences across two experimental groups was calculated using unpaired two-tailed Student's *t*-test in GraphPad Prism 6.0 software. A p-value <0.05 (*) was considered statistically significant. All data are shown as mean±SD (otherwise indicated in the figure legend) of one representative experiment of at least three independent experiments showing similar results.

Results

Tau expression in different prostate cancer cells and association between docetaxel resistance and tau in prostate cancer cells

To extend tau expression in different prostate cell lines, we examined three prostate cancer cell lines, LNCaP, PC-3, and DU145, from which DR clones were previously established.[1] Western blot was used to measure the expression of tau protein, and we found that abundant levels of tau protein were detected in DU145 cells, modest levels were found in PC-3 cells, and LNCaP cells showed no significant tau expression (Figure 1A). We further evaluated tau mRNA expression by qRT-PCR in prostate cancer cell lines and the resistant clones. We found higher levels of tau mRNA in DU145 cells and modest levels in PC-3 cells. On the contrast, LNCaP cells showed no significant tau mRNA (Figure 1B). Also, we found that DR PC-3 DR and DU145 DR expressed higher levels of tau than the parental cells (Figure 1C), suggesting that tau upregulation was related to biological resistance to docetaxel. Interestingly, in PC-3 cells and DU145 cells, higher levels of tau mRNA were found in the resistant clones compared with that in parental cells (Figure 1D). All these results suggest that tau might also play an important role in docetaxel resistance.

Inactivation of tau suppresses cell growth in prostate cancer cells

To determine if tau can be a diagnostic target in prostate cancer, we inactivated tau by constructing three plasmids using shRNAs (shTau-1, shTau-2, and shTau-3). The expression levels of tau knockdown were confirmed by western blot, and we found that tau knockdown, as compared to the scramble control (S-control), significantly reduced tau expression in naïve (parental) PC-3 and DU145 as well as in their corresponding DR clones, although the tau knockdown achieved in resistant cells was not as pronounced as in the naïve cells (Figure 2A). Tau mRNA expression after tau knockdown was observed by qRT-PCR. We also found that tau mRNA expression in transfected cells (shTau-1, shTau-2, and shTau-3) was lower than that of scramble control (Figure 2B). However, inactivation of tau dramatically inhibited proliferation of naïve and DR PC-3 and DU145 (Figure 2C), indicating that tau protein may be considered as a diagnostic target of prostate cancer.

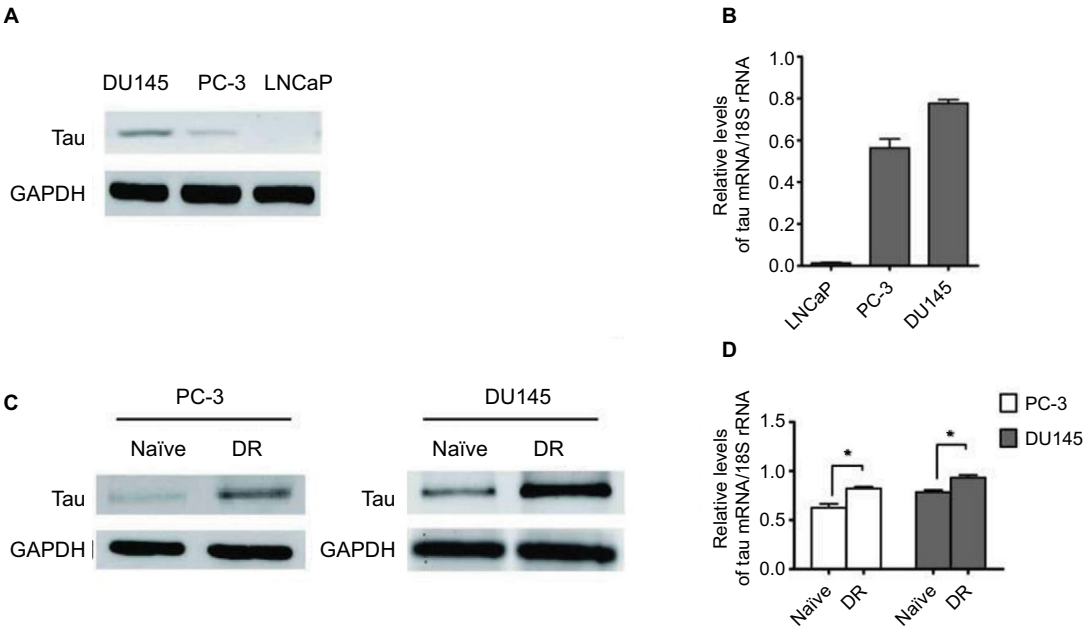

Figure 1 Expression of tau in primary and recurrent prostate cancer cell lines.
Notes: Protein (**A**) and mRNA (**B**) expression of tau in prostate cancer cell lines. Protein (**C**) and mRNA (**D**) expression of tau in PC-3 and DU145-naïve and DR cells. Data are shown as mean±SD. *$p<0.05$. Results are shown as mean±SD.
Abbreviations: DR, docetaxel-resistant; SD, standard deviation.

Inactivation of tau enhanced docetaxel sensitivity in prostate cancer cells

We then detected the effect of tau knockdown on docetaxel sensitivity. We used shRNAs to knockdown tau. We found that inactivation of tau protein could enhance the sensitivity of prostate cancer cells to docetaxel, the microtubule targeting agent, and shTau-2 has shown a greater inhibitory effect than shTau-1 and shTau-3 (details data not shown).

The effect of inactivation of tau on docetaxel-induced cytotoxicity was further compared between DR and naïve cells. We found that treatment with shTau resulted in a distinct shift of the docetaxel IC_{50} values in both PC-3 DR and DU145 DR cells compared with naïve PC-3 and DU145 cells, which suggested that DR cells were more sensitive to the combined inactivation of tau and docetaxel treatment than their parental counterparts (Figure 3A, B). These results indicated that MAPT can be a therapeutic target in the treatment for prostate cancer.

PI3K/Akt/mTOR signal pathway associated with the resistance to docetaxel in prostate cancer cell lines

The mechanisms through which tau mediate docetaxel resistance remain unknown. Recently, the PI3K/Akt/mTOR pathway has played an essential role in the regulation of cell growth, division, and tumorigenesis.[10] Especially, the PI3K/Akt/mTOR pathway is associated with resistance to multiple chemotherapeutic drugs and inactivating this pathway rendered prostate cancer cells more sensitive to chemotherapeutic drugs targeting microtubules such as paclitaxel, vinblastine, and docetaxel.[11] Therefore, by investigating PI3K/Akt/mTOR pathway on DR prostate cancer cells and its parental cells, we attempted to clarify whether this signaling pathway is associated with docetaxel resistance. As shown in our results (Figure 3C), the PI3K/Akt/mTOR pathway was upregulated in DU145 DR cells compared with the DU145-naïve cells, which indicated that this signaling pathway is associated with docetaxel resistance. Furthermore, since the tau protein has been confirmed to be associated with resistance to docetaxel in prostate cancer cells, these studies prompted us to shed light on the PI3K/Akt/mTOR signal pathway whereby tau protein is associated with resistance to docetaxel in prostate cancer cell lines. To our result, inactivating expression of tau significantly downregulated the expression of p-mTOR, p-4EBP1, and p-S6K but slightly downregulated the expression of Akt (Figure 3C), indicating that MAPT is associated with the resistance to docetaxel in prostate cancer cell lines by regulating the PI3K-signaling pathway. Probably, however, further experiment would be performed to confirm this conclusion. Thus, targeting tau protein and PI3K/Akt/mTOR signal pathway is a promising strategy to enhance docetaxel response for the treatment of prostate cancer.

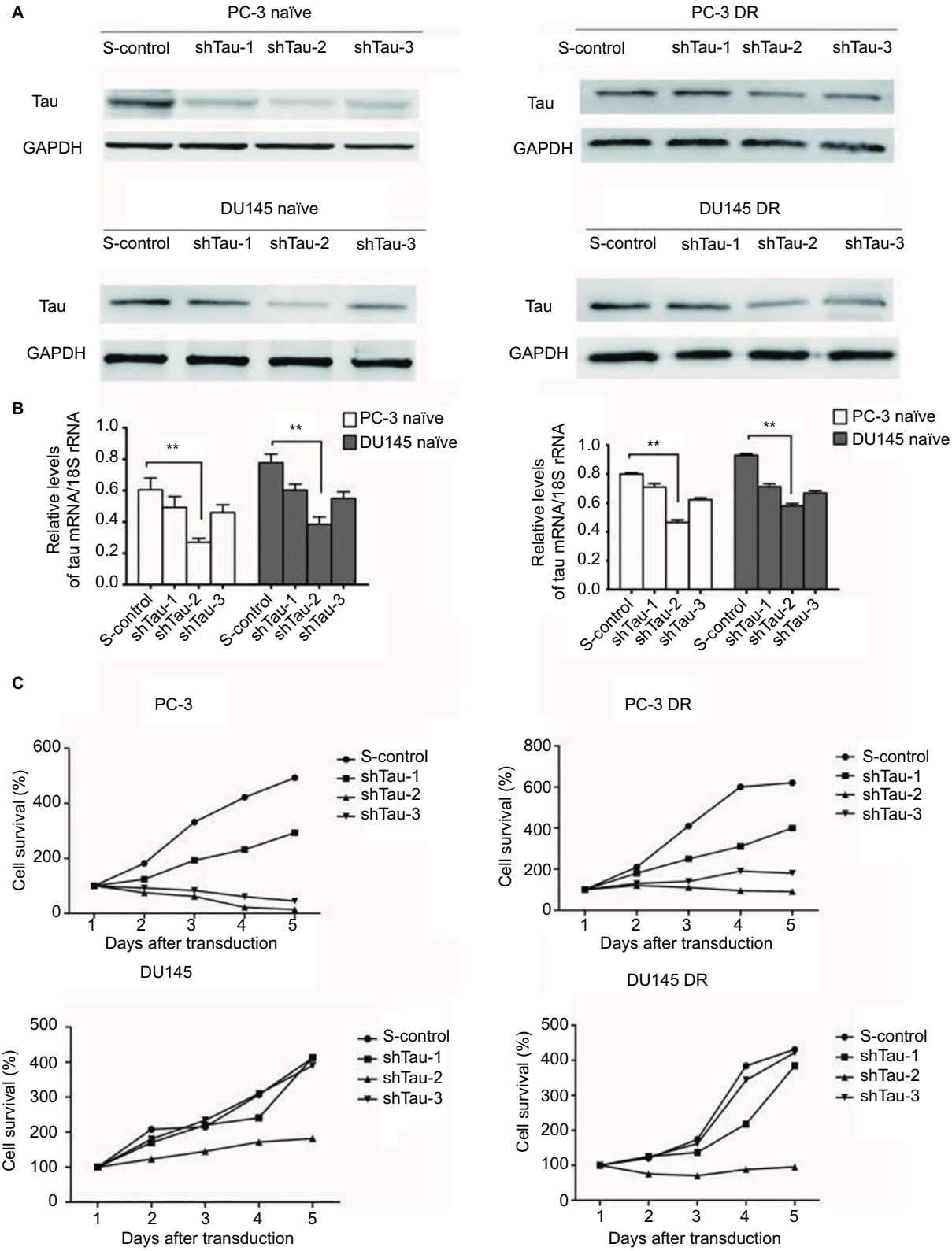

Figure 2 Tau knockdown reduces prostate cancer cell growth.

Notes: (**A**) Immunoblot shows tau knockdown efficiency 48 h after transduction with lentivirus expressing tau shRNAs as compared to scramble control (S-control) shRNA (pLKO.1) in PC-3 and DU145-naïve and docetaxel-resistant (DR) cells. GAPDH was used as the loading control. (**B**) mRNA expression of tau after transduction with lentivirus expressing tau shRNAs as compared to control shRNA (pLKO.1) in PC-3 and DU145-naïve and DR cells. $^{**}p<0.01$. Results are shown as mean±SD. (**C**) The effect of tau knockdown on cell proliferation. Cell numbers are determined daily.

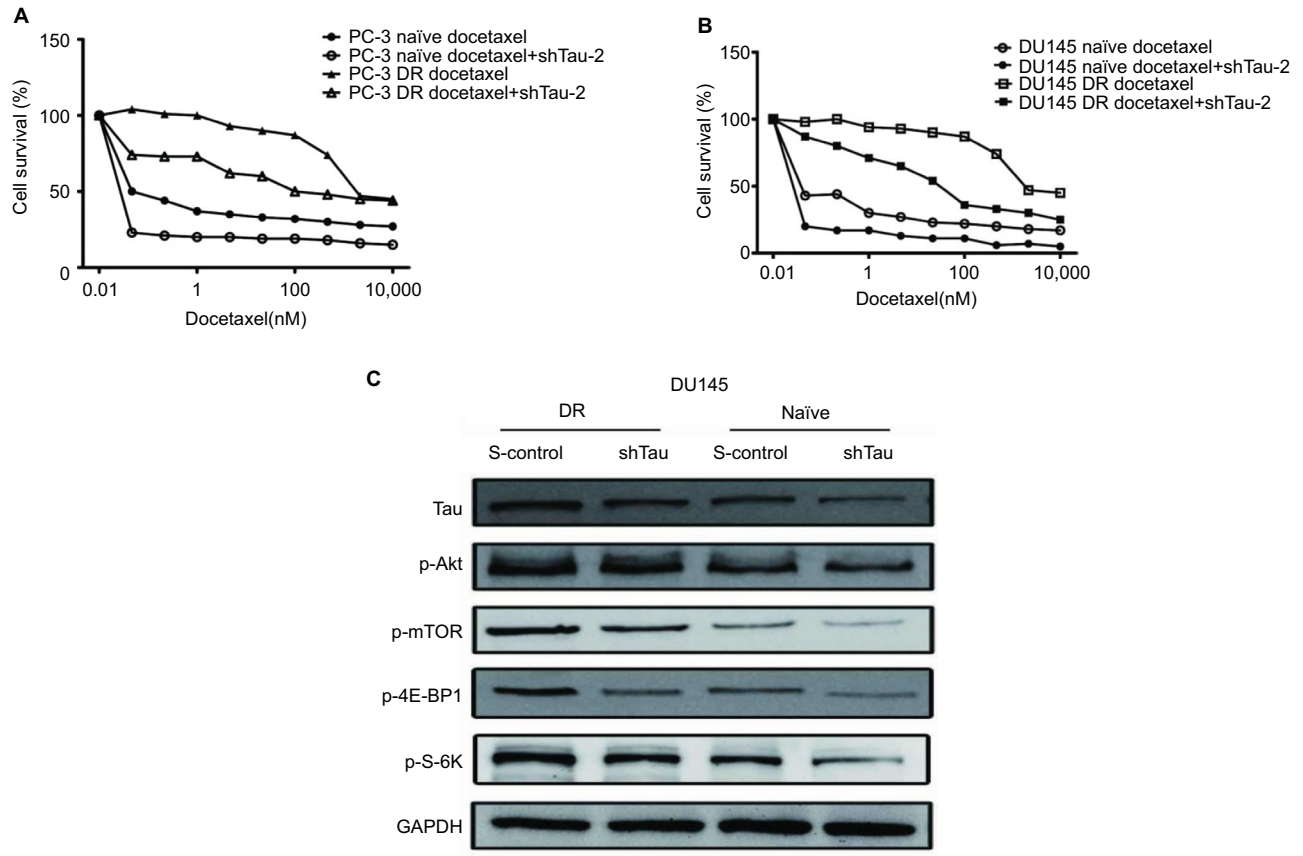

Figure 3 Combination of tau knockdown and docetaxel produces a synergistic cytotoxicity and PI3K/Akt/mTOR signaling pathway is involved**.**
Notes: Cell viability assay showing the effect of addition of tau knockdown to docetaxel in DR cells as compared to naïve PC-3 cells (**A**) or DU145 cells (**B**). Results are shown as ±SEM. (**C**) Levels of p-Akt, p-mTOR, p-4EBP1, and p-S6K in naïve or DR DU145 cell line treated with tau knockdown or scramble control (S-control), compared to control. GAPDH was used as the loading control. p-Akt: phosphorylated-Akt.
Abbreviations: DR, docetaxel-resistant; p-Akt, phosphorylated-Akt; p-mTOR, phosphorylated-mTOR; p-4EBP1, phosphorylated-4EBP1; p-S6K, phosphorylated-S6K.

Discussion

Here, we report the initial functional characterization of the MAPT, which is broadly and abundantly expressed in prostate cancer cells and is associated with the resistance to docetaxel in prostate cancer cells. It is much likely that the DR cells with higher tau levels and activity have set up a stable molecular mechanism that neutralizes docetaxel-mediated microtubule stabilization, thus lessening the docetaxel-induced cytotoxicity.

Tau has been proved to play a significant role in Alzheimer's disease.[6] In addition, the abnormal expression of tau protein was previously reported in many cancers, and the role of tau in cancer treatment has also been reported by others, such as gastric carcinoma,[13,14] breast cancer,[12,15,16] and ovarian cancer.[9,17]

It is well known that microtubules have been demonstrated as a crucial target of chemotherapeutic drugs for molecular cancer therapeutics. The classification of antimitotic microtubule-targeting drugs include 1) vinca alkaloids (such as vinblastine, vincristine, vindesine, and vinorelbine) that bind to α- and β-tubulin, restraining their combination to microtubules and microtubule polymerization, and 2) taxanes (such as paclitaxel and docetaxel) that bind to microtubule, directly interacting with and stabilizing microtubules.[18,19]

Tau inactivation and docetaxel treatment may explain their synergistic effect in treatment for tumors, especially in those that have docetaxel resistance, such as prostate cancer.

To our result, substantial levels of tau protein were detected in prostate cancer cells, and DR cells expressed higher levels of tau than the parental cells, which indicated that tau might also play an important role in docetaxel resistance and it can be a diagnostic target in prostate cancer. What's more, co-treatment of tau knockdown and docetaxel may explain their synergistic effect in treatment for tumors. Therefore, the tau inhibitor was expected to be found out to increase the docetaxel sensitivity for the treatment of prostate cancer. In conclusion, MAPT can be a therapeutic target in prostate cancer.

The PI3K/Akt/mTOR pathway plays an essential role in the regulation of cell growth, division, and tumorigenesis.

Our study clarified that resistance to docetaxel in prostate cancer cells is associated with PI3K/Akt/mTOR pathway. However, tau protein associated with the resistance to docetaxel in prostate cancer cell lines via the PI3K/Akt/mTOR signaling pathway needs more powerful evidence.

In conclusion, our results may have significant clinical progress in tumor chemotherapeutics because docetaxel is frequently used in treating a series of major types of cancers such as lung and breast carcinoma besides prostate carcinoma. However, because of the development of docetaxel resistance, introduction of countermeasures to conquer docetaxel resistance is an imperative requirement for prostate cancer.

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Disclosure

The authors report no conflicts of interest in this work.

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