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LETTER TO THE EDITOR

Prostate Disease

Autophagy: a stumbling block of androgen inhibition to treat benign prostatic hyperplasia or prostate cancer

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Dear Editor,

Benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are the two most common prostatic disorders affecting elderly males and represent significant burdens for health-care systems worldwide.[1] BPH and PCa exhibit important differences in terms of histology and localization, and to date, no causal relationship between the two prostatic diseases has been demonstrated, but BPH and PCa indeed share traits such as androgen-dependent growth and response to hormonal therapy.[2] Over the past decades, introducing anti-androgen drugs (*e.g.,* 5α reductase inhibitors, 5-ARI for BPH and CYP17 inhibitors for PCa) to treat the two prostatic diseases has offered remarkable benefits.[2] Whereas, relatively little attention has been paid to whether the sole administration of anti-androgen drugs is appropriate or sufficient to obtain the maximum therapeutic effect against BPH or PCa. Thus, we write this letter to draw attention to the importance of reevaluating the effective pharmacological activity of anti-androgen drugs in treating BPH or PCa. Specifically, we will provide some recent advances exploring synergistic interventions to treat BPH or PCa via modulating autophagy, an adaptive process that enables cells to cope with metabolic, toxic and other stressors.

AUTOPHAGY AS A MODULATOR IN ANTI-ANDROGEN THERAPY TO BPH

A recent laboratory investigation containing some clinical data from Li *et al.*[3] suggested a novel strategy to achieve better therapeutic efficacy in 5-ARI-treated BPH via autophagy inhibition and apoptosis induction. The study showed an accumulation of autophagosomes ($P < 0.001$) following dihydrotestosterone (DHT) deprivation in PWR-1E prostatic epithelial cells, indicating autophagy was elicited during androgen inhibition. *In vivo* immunohistochemistry (IHC) staining was performed on human prostate tissues from BPH patients to investigate the role, if any, of autophagy in 5-ARI treatment. The results showed the LC3 expression level was higher in the 5-ARI-treated group than the control group (25.36 *vs* 14.19, $P < 0.01$). In addition, the basal apoptosis rate of PWR-1E cells under androgen deprivation (AD) was 2.52% ± 1.35%, after treatment with specific autophagy inhibitor 3-methyladenine (3-MA) for 24 h, the apoptosis rate in AD condition dramatically increased to 7.18% ± 2.44% ($P < 0.001$), which could contribute to the volume shrinkage of the prostate effectively. Altogether, the results suggested the autophagy blockade might be a promising approach to reducing more prostate volume via apoptotic activity enhancement in prostatic epithelial cells. This work is technically sound and demonstrates the effectiveness of a combination therapy for treating BPH using 5-ARI and an autophagy inhibitor for the first time. In addition to this study, Liu *et al.*[4] divided 96 paraffin-embedded BPH tissue samples into 5α-reductase inhibitor administration group and matched control group. Through careful IHC analysis, they found that the autophagic core machineries Beclin-l and LC3 expression in the patients who had taken 5α-reductase inhibitor were significantly higher compared with that in the control group ($P < 0.05$), verifying the elevated autophagy activity after androgen ablation.

AUTOPHAGY AS A MODULATOR IN ANDROGEN DEPRIVATION THERAPY TO PCA

As mentioned before, BPH and PCa are both responsive to androgen, primarily testosterone (T) and dihydrotestosterone (DHT).[2] Thus, it follows that both BPH and PCa could further benefit from a combination of androgen deprivation and autophagy inhibition therapy. In 2008, Li *et al.*[5] addressed that androgen-sensitive prostate cancer LNCaP cells could survive under androgen deprivation conditions by resorting to autophagy induction. Moreover, autophagy depression by a pharmacological inhibitor, such as 3-MA or small interfering RNA targeted to Beclin-1 (one canonical autophagy-related gene), could result in dramatically increased apoptotic activity and decreased cell viability of LNCaP cells while under androgen deprivation condition compared with the culture medium contained androgen or serum ($P < 0.05$). The anti-malarial drug chloroquine (CQ), which is known to increase the lysosomal pH and mitigate acidic degradation, is presently undergoing clinical trials and appears to be a promising therapeutic for several cancers including prostate cancer.[6] In 2012, Kaini and Hu[7] observed that CQ synergistically killed LNCaP cells in a time- and dose-dependent manner during androgen deprivation by reducing the cytosolic ATP level and inducing nuclear condensation leading to DNA fragmentation and ultimately apoptosis. This is corroborated

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by Boutin *et al*.[8] who elucidated that removal of an androgen receptor (AR) agonist (R1881) or treatment with a nonsteroidal AR antagonist (bicalutamide) could elicit autophagy in LNCaP cells, which process was dependent on Atg5 and Beclin-1 through the mTOR signaling cascade and ultimately led to a dramatic reduction in the phosphorylated forms of p70S6k and Akt. Notably, bicalutamide indeed increased apoptosis-associated cell death dramatically after blocking autophagy via genetic knockdown of Atg5 or pharmacological inhibitor CQ treatment, as increased sub-G1 cell population, mitochondrial transmembrane potential ($\Delta\Psi m$) dissipation, and plasma membrane permeabilization were observed by DNA content analysis and $\Delta\Psi m$-sensitive probe $DiOC_6$ (3) labeling, respectively. To clarify the modulatory role of autophagy in BPH and PCa tissues during androgen deprivation or antagonism more clearly, we have summarized the representative studies which were designed to elucidate the issue *in vitro* or *in vivo* (**Table 1**).

AUTOPHAGY IS A DOUBLE-EDGED SWORD IN CELL FATE DETERMINATION

Autophagy has been implicated in various physiological and pathological processes. Intriguingly, autophagy often localizes to metabolically stressed regions and is thus regarded as a controversially double-edged sword in cell fate determination. For one thing, autophagy plays a vital role in cell damage augment, which ultimately causes or mediates autophagic cell death. For another, autophagy protects cells from formidable conditions including starvation, growth factors deficiency (which is specifically referred to as androgen deprivation in BPH and PCa), and even medical interventions such as chemotherapy or radiotherapy. A phase II randomized, double-blind, placebo-controlled clinical study endeavored to show that patients with metastases throughout the brain could had a greater therapeutic outcome from a combination of irradiation and treatment with CQ leading to increased progression-free survival of brain metastases (BMPFS) at 1 year from 55.1% (95% CI 33.6–77.6) to 83.9% (95% CI 69.4–98.4).[9] Even still, autophagy was erroneously presumed to be merely a cell death pathway and often execute autophagic cell death directly. The present studies highlighted that one of the major functions of autophagy is to assist cells (including hyperplastic or cancerous prostatic cells) in adapting to various "life-threatening" situations such as androgen deficiency and also prevent their apoptotic activities, in consideration of the extensive crosstalk between autophagy and apoptosis which enables the coregulation of cell fate determination.[10] This is crucial for designing therapeutic approaches for both BPH and PCa, especially when considering several studies have addressed the definitely pro-survival role of autophagy during androgen inhibition.

SUMMARY AND PERSPECTIVES

The aforementioned investigations presented multiple lines of evidence to conclude that genuine autophagy was induced during androgen inhibition and subsequently generated the "escaping effect" from apoptosis in prostatic epithelial cells and cancer cells, which taken together impaired the pharmacological efficacy in achieving maximal prostate volume shrinkage or PCa destruction. Notably, several clinical trials relevant to autophagy inhibition in prostate cancer therapy (mostly by chloroquine or its analogue hydroxychloroquine co-administration) have been carried out or under recruiting participants, such as "Akt inhibitor MK2206 and hydroxychloroquine in treating patients with advanced solid tumors or prostate or kidney cancer (Phase I, Identifier: NCT01480154)" and "hydroxychloroquine in treating patients with rising PSA levels after local therapy for prostate cancer (Phase II, Identifier: NCT00726596)." Moreover, a phase II study of ABT-263/abiraterone or ABT-263/abiraterone/hydroxychloroquine in prostate cancer (Phase II, Identifier: NCT01828476) has shed light on the "proof-of-concept" synergistic therapy for PCa via androgen deprivation plus autophagy inhibition.[10] Conclusively, all the valuable preclinical and clinical results revealed that simultaneous androgen deprivation and autophagy inhibition might hold much greater promise than either therapy alone for patients with BPH or PCa.

AUTHOR CONTRIBUTIONS

LZ and JZ conceived of the study, LZ drafted the manuscript and JZ revised it. SF participated in the design of the study. CZL participated in the design and coordination and critical revision of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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Table 1: The role of autophagy in benign prostatic hyperplasia and prostate cancer cell fate determination after anti-androgen treatment

Authors	*Anti-androgen*	*Elevated autophagy*	*Autophagy inhibitor*	*Apoptotic cell death after autophagy inhibition*	*Cell type and tissue*
Li *et al*.[3]	5-ARI	LC3 expression↑	3-MA	Apoptosis rate↑	PWR-1E prostate epithelial cells, BPH tissues
Liu *et al*.[4]	5-ARI	LC3 expression↑ Beclin-1 expression↑	NA	NA	BPH tissues
Li *et al*.[5]	Androgen deprivation	LC3 expression↑ Beclin-1 expression↑ MDC-positive cells↑ Autophagosomes↑	3-MA Beclin-1 siRNA	Apoptotic cells↑ (PI-positive cells)↑	LNCaP prostate cancer cells
Kaini and Hu[7]	Androgen deprivation	Autophagic vesicles↑	CQ	Cytosolic ATP↓ Nuclear condensation↑ DNA fragmentation↑	LNCaP prostate cancer cells
Boutin *et al*.[8]	R1881/SSH removal Bicalutamide	LC3 expression↑ p62/SQSTM1↓ Autophagosomes↑ Phospho-p70S6k↓ Phospho-Akt↓	CQ Atg5 siRNA	Sub-G1 cells↑ PI-positive cells↑ $DiOC_6(3)^{low}$ cells↑	LNCaP prostate cancer cells

5-ARI: 5α reductase inhibitor; LC3: microtubule-associated protein light chain 3; BPH: benign prostatic hyperplasia; 3-MA: 3-methyladenine; CQ: chloroquine; MDC: monodansylcadaverine; PI: propidium iodide; ATP: adenosine triphosphate; R1881: methyltrienolone; SSH: serum steroid hormone; Akt: protein kinase B; p70S6k: 70 kDa ribosomal protein S6 kinase; Atg5: autophagy-related gene 5; $DiOC_6(3)$: mitochondrial membrane potential ($\Delta\Psi m$)-sensitive fluorochrome; NA: not analyzed

REFERENCES

1 Ørsted DD, Bojesen SE. The link between benign prostatic hyperplasia and prostate cancer. *Nat Rev Urol* 2013; 10: 49–54.
2 Wang K, Fan DD, Jin S, Xing NZ, Niu YN. Differential expression of 5-alpha reductase isozymes in the prostate and its clinical implications. *Asian J Androl* 2014; 16: 274–9.
3 Li MQ, Yang X, Wang HL, Xu EC, Xi ZJ. Inhibition of androgen induces autophagy in benign prostate epithelial cells. *Int J Urol* 2014; 21: 195–9.
4 Liu CY, Xu PC, Chen DG, Fan XH, Xu YP, *et al*. Roles of autophagy-related genes Beclin-1 and LC3 in the development and progression of prostate cancer and benign prostatic hyperplasia. *Biomed Rep* 2013; 1: 855–60.
5 Li MQ, Jiang XJ, Liu D, Na YQ, Gao GF, *et al*. Autophagy protects LNCaP cells under androgen deprivation conditions. *Autophagy* 2008; 4: 54–60.
6 Vyas AR, Hahm ER, Arlotti JA, Watkins S, Stolz DB, *et al*. Chemoprevention of prostate cancer by d, l-sulforaphane is augmented by pharmacological inhibition of autophagy. *Clin Cancer Res* 2013; 73: 5985–95.
7 Kaini RR, Hu CA. Synergistic killing effect of chloroquine and androgen deprivation in LNCaP cells. *Biochem Biophys Res Commun* 2012; 425: 150–6.
8 Boutin B, Tajeddine N, Vandersmissen P, Zanou N, Van Schoor M, *et al*. Androgen deprivation and androgen receptor competition by bicalutamide induce autophagy of hormone-resistant prostate cancer cells and confer resistance to apoptosis. *Prostate* 2013; 73: 1090–102.
9 Rojas-Puentes LL, Gonzalez-Pinedo M, Crismatt A, Ortega-Gomez A, Gamboa-Vignolle A, *et al*. Phase II randomized, double-blind, placebo-controlled study of whole-brain irradiation with concomitant chloroquine for brain metastases. *Radiat Oncol* 2013; 8: 209–17.
10 Farrow JM, Yang JC, Evans CP. Autophagy as a modulator and target in prostate cancer. *Nat Rev Urol* 2014; 11: 508–16.