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An Invited Review Following *the Soujinkai Fujiu Memorial Award*: Elucidation of Drug Resistance Mechanisms in Prostate Cancer: Overcome Them by Investigating Bench to Bedside

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Abstract Androgen receptor (AR)-signaling plays an important role in promoting tumor cell survival and the development of castration-resistant prostate cancer (CRPC). Androgen deprivation therapy is the basic therapeutic strategy for AR signaling blockade, but that effects won't last long and most of metastatic prostate cancer show castration resistance. Recently, new agents, AR Axis Targeted agents (ARAT), have been developed. We have been developing our bench-to-bedside translational research to elucidate the mechanisms of drug resistance in prostate cancer. This review describes our investigational results and recent advances in CRPC with ARAT treatment strategy.

Key words: castration-resistant prostate cancer, androgen receptor, clusterin, castration-sensitive prostate cancer, AR axis targeted agent

Introduction

In recent years, the development of new therapeutic targets for castration-resistant prostate cancer (CRPC) has been rapid, and it is likely to change dramatically in the future with chemotherapy, molecular targeted therapy, and modulation of cancer immuno-microenvironment-based therapy.[1] However, in prostate cancer, androgen receptor (AR) targeted therapy is still the mainstay of treatment. Blockade of the AR signaling pathway is one of the most important therapeutic strategies in prostate cancer, and it has been shown that AR still plays a major role in cancer survival even in CRPC.[2] Abiraterone, enzalutamide (MDV3100), Apalutamide and Darolutamide are developed to be the next generation of AR Axis Targeted agent (ARAT).[3-5] However, the stress on cancer cells caused by AR signal blockade, anticancer drugs, and radiation therapy is known to activate clusterin (CLU) and other chaperone molecules, mainly CLU cause resistance to treatment, which has been suggested to be a dilemma in overcoming prostate cancer.[6] We have previously reported that castration, anticancer drugs, and radiation therapy increase the expression of CLU in prostate cancer, which leads to resistance to treatment.[7-10]

While, AR and its cochaperone is also important to overcome castration resistance. Then we have tried to conquer the castration resistance by another approach which is reproducing cancer microenvironment with 3D culture material. And we have developed bone microenvironment by coculture of cancer cells and osteoblasts for mimicking bone metastasis in prostate cancer. Moreover, we have been corroborating other faculties and we have been searching new drug targets for CRPC.

In clinical field, We have participated in international clinical trials and played a role in the launch of ARAT, enzalutamide.

In this minireview, we describe the molecular mechanisms of acquisition of castration resistance in our basic research and therapeutic challenges to be overcome in prostate cancer.

From bench

Based on the results of previous basic research, the University of British Columbia and Oncogenex developed OGX-011 (Custirsen), an antisense inhibitor of CLU, and conducted a phase II study in CRPC patients.[11] While, emerging second generation anti androgen drug, Enzalutamide (MDV3100) has shown to be clinical usefulness for CRPC, but its efficacy doesn't keep long and CRPC patients will eventually become resistant to enzalutamide.[12,13]

Based on the above facts, we are conducting in vitro and in vivo experiments to confirm whether the combination of AR inhibitors and CLU knockdown has a synergistic effect and we are attempting to elucidate the molecular mechanism that causes the synergistic effect and to apply it to therapy.

We established a castration-resistant mouse model and demonstrated the possibility of overcoming treatment resistance proving the growth mechanism mediated by androgen receptor signaling pathway with the cellular level. We have demonstrated the possibility of overcoming therapeutic resistance at the animal level. We have found that AR suppression results in a high degree of enzalutamide resistance and castration resistance at the cellular level and in mouse models. Enzalutamide also caused activation of CLU as well as activation of AKT and MAPK signaling pathways. The mechanism of this stress response is partly mediated by a feed-forward loop in which CLU induced by YB-1 phosphorylation activates p90rsk, which in turn promotes YB-1 phosphorylation. In a castration-resistant mouse model of subcutaneous implantation of the prostate cancer cell line LNCaP, the combination of Enzalutamide and OGX-011 was shown to synergistically promote apoptosis, suppress PSA recurrence and inhibit tumor growth by simultaneously inhibiting AR and CLU[14] (Fig. 1-4). These results indicate that the combination of AR inhibition and CLU control is theoretically useful and a feasible therapeutic strategy for clinical trials.[11,15,16]

Unfortunately, in phase 2 and 3 trial of CLU combination with docetaxel and cabazitaxel, we could not show significant synergistic effect in CRPC,[17,18] then Custirsen development has been finished in prostate cancer.

For next step, in terms of competitive funding, we were awarded a grant of KAKENHI project (16K11008) for "Development of the mechanism of progression of castration-resistant prostate cancer to neuroendocrine carcinoma and drug-selective markers" and a New Frontier Research Grant in 2016, and succeeded in establishing the first cancer organoids from a prostatectomy whole specimen.[19] In addition, we analyzed the relationship between YAP and its regulator ARHGAP29 in prostate cancer cells in 3D culture, and reported the mechanism of prostate cancer progression by ARHGAP29. Immunostaining of whole prostatectomy specimens showed that YAP and ARHGAP29 were significantly correlated with clinicopathological factors and prognosis. Cell line analysis showed that the expression of ARHGAP29 downstream of YAP was predominantly upregulated in PC3 cells and significantly downregulated in LNCaP cells. Knockdown of ARHGAP29 in PC3 cells significantly inhibited cell proliferation, decreased invasive capacity, and promoted phosphorylation of YAP. Conversely, forced expression of ARHGAP29 in LNCaP cells promoted cell proliferation and significantly enhanced invasive capacity. In addition, knockdown of ARHGAP29 in PC3 cells increased phosphorylation of Cofilin, indicating that the YAP-ARHGAP29-Cofilin pathway is involved in the acquisition of malignant traits in prostate cancer.[20]

And also, we focused of reproduction of bone microenvironment mimic to bone metastasis of CRPC, we established in-vitro model by chitosan nanofiber matrix-coated culture plates to simulate the 3D scaffold of the bone microenvironment, in co-culture with cancer cells and osteoblasts derived from human bone marrow monocyte. Combination treatment with abiraterone and

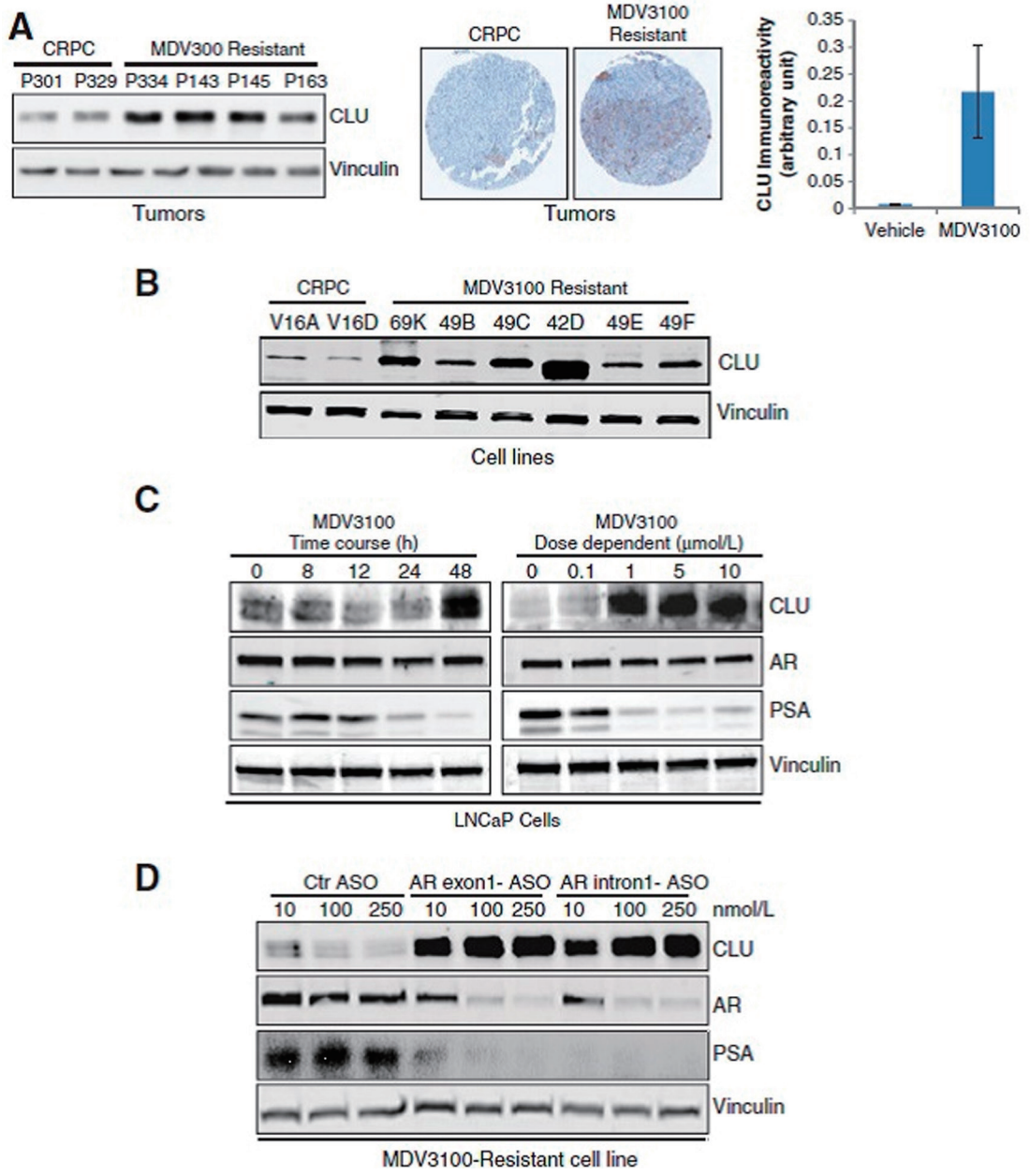

Fig. 1

MDV3100 induces upregulation of CLU. A, CLU is highly expressed in MDV3100-resistant xenografts. Western blot analysis was conducted from MDV3100-resistant or control CRPC tumors using CLU and vinculin (as a loading control) antibodies (left). Tumors were evaluated by immunohistochemistry using CLU antibody on MDV3100-resistant tumors and control CRPC tumors (middle, right). B, CLU is highly expressed in MDV3100-resistant cell lines. Western blot analysis was conducted from cell lines generated from CRPC and MDV3100-resistant tumors using CLU and vinculin antibodies. C, CLU is induced by MDV3100 in time- and dose-dependent manner. LNCaP cells were treated with MDV3100 at indicated time (left) or concentration (right) and Western blot analysis was conducted with CLU, AR, and PSA antibodies. D, CLU was induced after AR knockdown LNCaP cells were treated with AR-ASO targeting AR exon 1 or intron 1, or control ASO and Western blotted for CLU, AR, and PSA antibodies.

*MDV3100: enzalutamide, CLU: clusterin, CRPC: castration resistant prostate cancer, AR: androgen receptor.

dutasteride synergistically inhibited the growth of C2-4 (CRPC cell line) colonies compared with individual investigational agents. This could be attributed to the reduction of 3-keto-5α-abiraterone which is known to act an androgen receptor agonist. The bone microenvironment model of the present study is unique and useful for evaluating new drug susceptibility testing in prostate cancer cells. This model may help to reveal the unknown mechanisms underlying micro- to clinical bone metastasis in prostate cancer.[21]

Aim to further development, as a co-principal investigator of the Yamaguchi University Center of Excellence Project "Elucidation of Cancer Growth Regulation and Establishment of Innovative Therapies" (Joint Faculty of Veterinary Medicine, Prof. Midori Shimada), we are continuing to analyze castration-resistant prostate cancer, and Chk1,2, FKBP family and drug resistance mechanisms and development of new target drugs are in progress.[22]

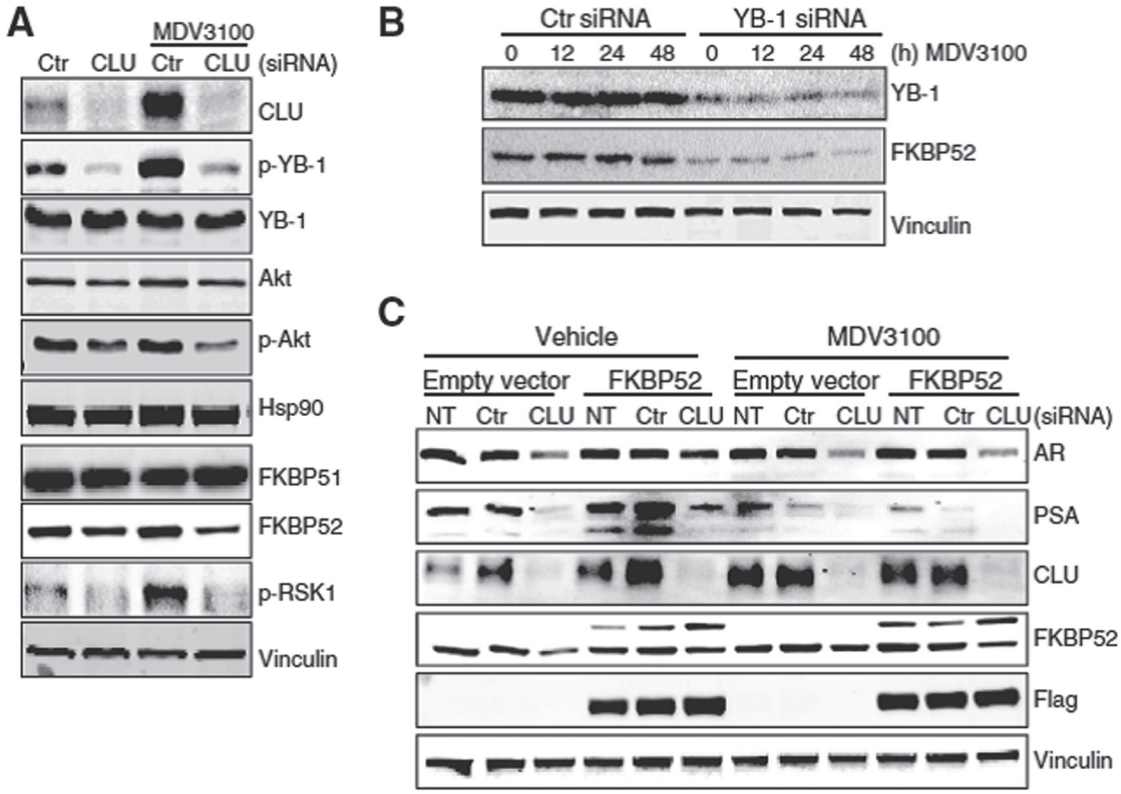

Fig. 2

CLU knockdown decreases FKBP52 levels. A, CLU knockdown decreases MDV3100-induced AKT and MAPK activation as well as FKBP52 levels.

LNCaP cells were transfected with CLU or control siRNA and treated with 10 mmol/L MDV3100 for indicated times. Proteins were extracted for Western blot analysis using indicated antibodies with actin used as loading control. B, YB-1 knockdown decreases FKBP52 expression. LNCaP cells were transfected with YB-1 or control siRNA and treated with 10 mmol/L MDV3100 for indicated times. Proteins were extracted for Western blot analysis using indicated antibodies. C, FKBP52 partially rescues AR degradation after CLU knockdown. LNCaP cells were transfected with FKBP52 Flag-tagged construct or empty vector followed by CLU knockdown. Forty-eight hours posttransfection, cells were treated with 10 mmol/L MDV3100 or vehicle for 24 hours. Proteins were extracted for Western blot analysis using indicated antibodies.

To bedside

Later, in 2014, enzalutamide was insured as a treatment for castration-resistant prostate cancer in Japan, and we participated in the phase III clinical trial (AFFIRM trial)[23] for its approval as an investigator. Enzalutamide group has shown significant efficacy with overall survival in post docetaxel CRPC patients (median survival time: enzalutamide arm 18.4 months vs placebo arm 13.6 months, hazard ratio 0.63: 95% CI, 0.53-0.75, P<0.001). I also participated in an international phase III trial for CRPC treated with pre-docetaxel setting (PREVAIL trial)[24] as a principal investigator. Enzalutamide group has shown significant increase of overall survival in pre-docetaxel CRPC patients (hazard ratio 0.71: 95% CI, 0.60-0.84, P<0.001). we also reported the results of the Japanese subgroup analysis as a co-investigastor.[25] Then now, enzalutamide is widely used for CRPC patients in the world and the research on resistance mechanisms against enzalutamide has also been conducted worldwide.

In a multi-center study, we analyzed the predictive factors for the therapeutic effect of enzalutamide and reported the association between testosterone levels and enzalutamide.[26]

In addition, an international clinical trial for the first-line treatment of metastatic castration-sensitive prostate cancer is currently published (ARCHES trail)[27] and recently Japanese subgroup analysis[28] has been done. Both studies showed enzalutamide group was significant prolongation of radiographic and

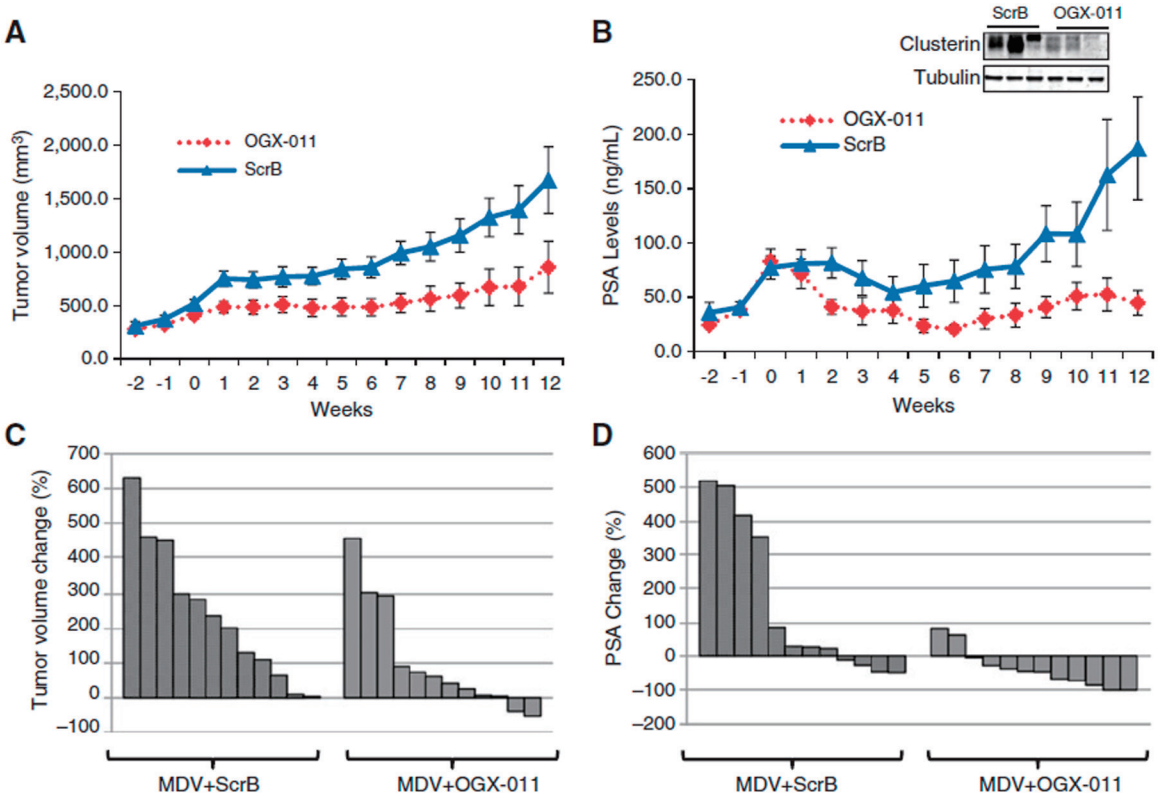

Fig. 3

OGX-011 enhances MDV3100 activity in CRPC LNCaP xenografts. Mice bearing CRPC tumors were randomized into 2 groups receiving 10 mg/kg MDV3100 daily plus either 10 mg/kg ScrB (12 mice) or OGX-011 daily for 1 week and then 3 times/week thereafter (12 mice). Mean tumor volume (A) and circulating PSA (B) for each treatment group is reported. Waterfall plots represent percent change in tumor volume (C) and PSA (D) at 12 weeks after treatment or at the point of sacrifice of mouse.

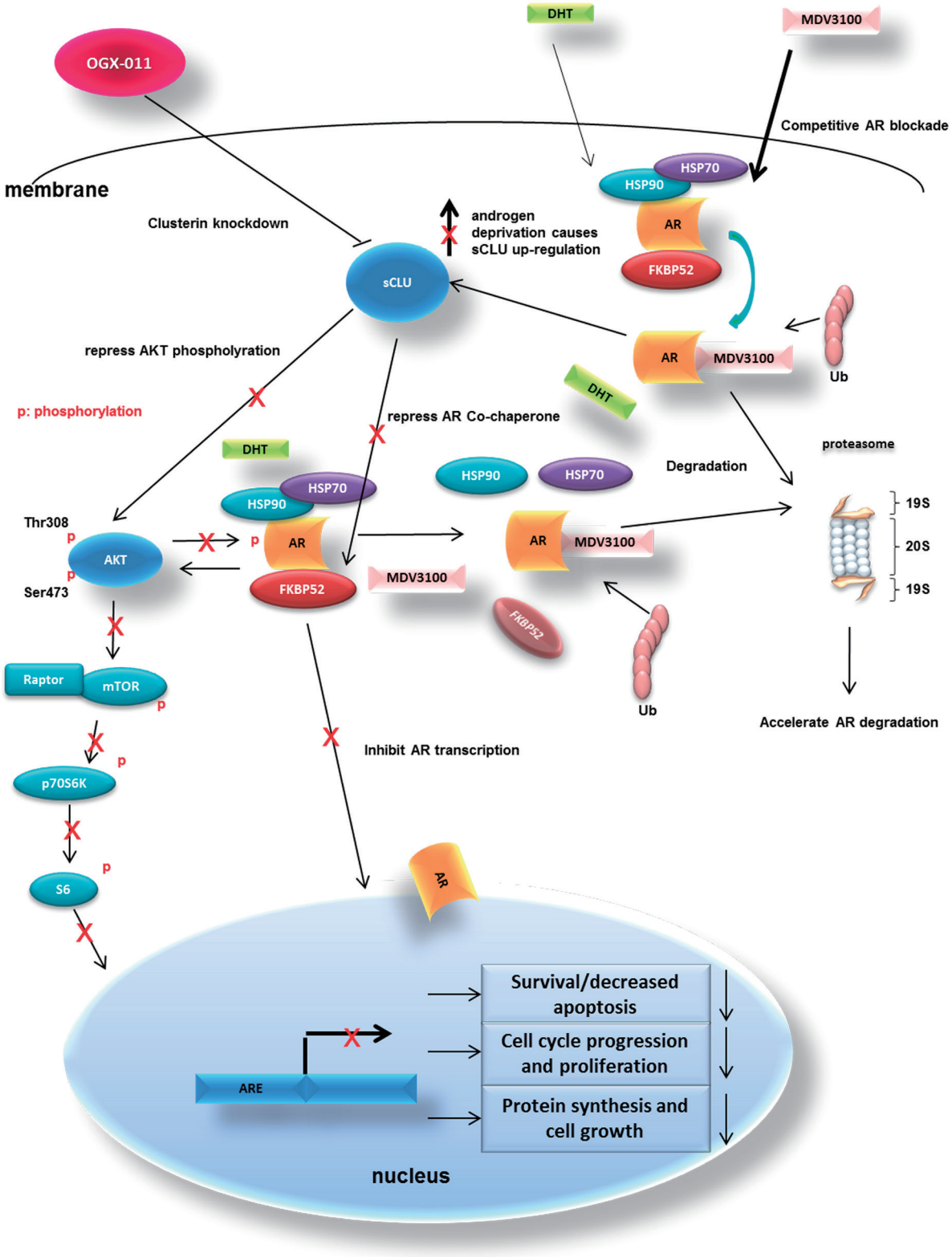

Fig. 4
The schema of putative mechanisms of synergy effect combined with OGX-011 and MDV3100 in CRPC

PSA progression free survival against placebo group. A series of studies are shown in the table (Table 1). For this, Japanese prostate cancer clinical practice guidelines will be revised next year.

Conclusions

It is still difficult to conquer CRPC, but I believe that series of achievements in translational research from basic research to clinical application, focusing on functional analysis of AR signaling pathway and cancer microenvironment give the principle of proof for development prostate cancer treatment. Further investigations are needed to conquer it.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1 The results of phase 3 study of development of enzalutamide in metastatic prostate cancer patients with castration sensitive or castration resistance

Clinical trial	setting	arm	Primary endpoint	Hazard ratio	P value	Secondary endpoint	Hazard ratio	P value
AFFIRM trial	CRPC post Doc	Enzalutamide 800 vs Placebo 399, 2:1	OS	0.63 (95%CI, 0.53-0.75)	p<0.001	rPFS	0.40 (95%CI, 0.35-0.47)	p<0.001
PREVAIL Trial	CRPC pre Doc	Enzalutamide 872 vs Placebo 845, 1:1	OS	0.71 (95%CI, 0.60-0.84)	p<0.001	Time to Chemo	0.35 (95%CI, 0.30-0.40)	p<0.001
ARCHES trial	CSPC	Enzalutamide 574 vs Placebo 576, 1:1	rPFS	0.39 (95%CI, 0.30-0.50)	p<0.001	OS*	0.66 (95%CI, 0.52-0.81)	p<0.001

OS: Overall survival, rPFS: Radiographic progression free survival, Time to Chemo: The time until the initiation of cytotoxic chemotherapy, Doc: Docetaxel, CRPC: castration resistant prostate cancer, CSPC: Castration sensitive prostate cancer.

*OS result was just reported at late braking session "LBA25 - Final overall survival (OS) analysis from ARCHES: A phase III, randomized, double-blind, placebo (PBO)-controlled study of enzalutamide (ENZA) + androgen deprivation therapy (ADT) in men with metastatic hormone-sensitive prostate cancer (mHSPC)" in ESMO Annual Congress 2021.

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