

REVIEW

# Regulation of Androgen Receptor by E3 Ubiquitin Ligases: for More or Less

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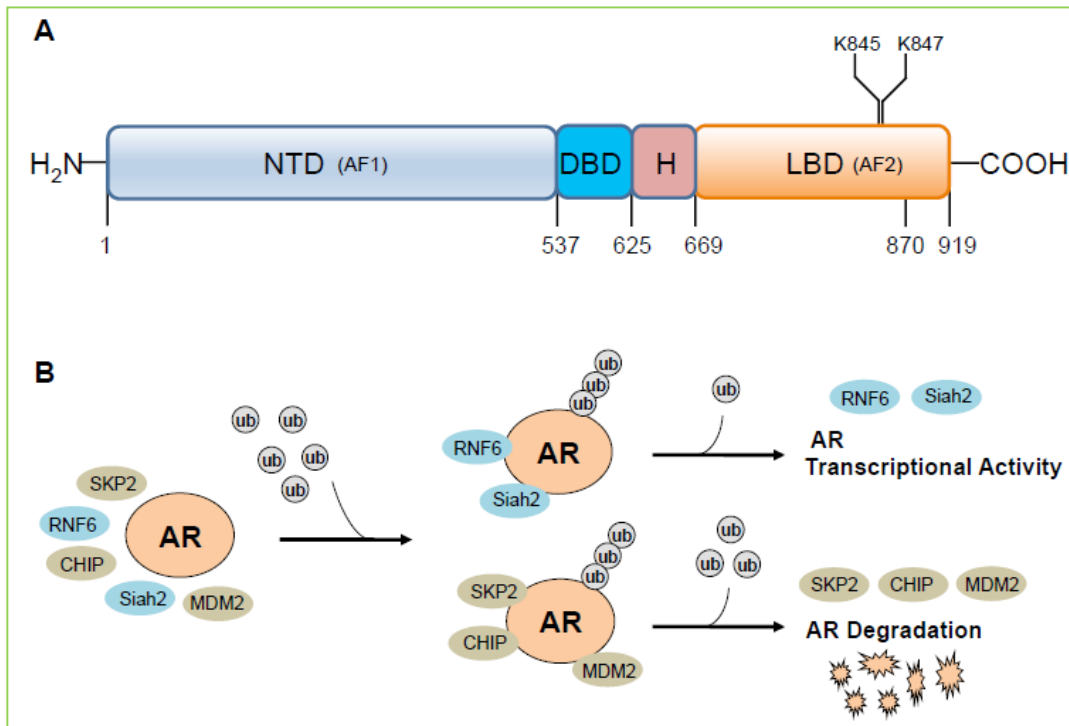
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## Introduction

Prostate cancer (PCa) primarily depends on the dysregulations of androgen receptor (AR) signaling pathway for the initiation and growth as well as recurrence after chemotherapy [1]. Androgen deprivation therapy (ADT) effectively alleviates symptoms of the malignancy to arrest further growth of primary tumors or progression of metastasis in patients with advanced PCa. However, relapse occurs in many patients after a short period, and PCa cells eventually become insensitive to ADT - termed castration resistant prostate cancer (CRPC) [2,3]. Tremendous advancements have been achieved to decipher the mechanisms on AR signaling, and the ubiquitination machinery contributes to PCa directly or indirectly by either promotion of AR transcriptional activity or degradation of AR protein levels. The recent report reveals that SKP2 regulates AR protein through ubiquitin-mediated proteasomal degradation, highlighting the role of SKP2 in AR signaling. Given the pivotal roles of AKT and SKP2 in cancers, the differential mechanisms of AR ubiquitination by various E3 ligases hold valuable significance and beneficial implications for PCa control.

Prostate Cancer (PCa) has long been a major health issue for men in developed countries such as nations in North America and Europe [4]. The essential role of androgen receptor (AR) signaling pathway is recognized for the morbidity and mortality of this malignancy because AR contributes to the maintenance of prostate function and further the initiation and development of PCa. In order to generate the roles in development, physiology and pathology, androgen ligands interact with AR that acts like a communication hub in the prostate cells to trigger molecular cascades. AR gene is located on X chromosome at Xq11-12<sup>[5,6]</sup>, and AR is a ligand-dependent nuclear receptor as the activity and function of AR are largely dependent on androgen. It is well-documented that AR signaling initiated by androgen-AR interaction contributes to a series of biological functions of prostates and molecular alterations leading to prostate carcinogenesis. Due to the coupling of androgen and AR, approaches to inhibit AR signaling pathway may be achieved through regulation of AR or androgen ligand. In two identified isoforms of AR protein, the full length form contains 919 amino acids, and the short form contains 732 amino acids lacking of 187 amino acids at N-terminal<sup>[7]</sup>. There are 4



**Figure 1. Androgen receptor (AR) is regulated by E3 ubiquitin ligases.** **A.** Diagram on the structure of AR protein. AR contains four domains, N-terminal domain (NTD), DNA binding domain (DBD), hinge region (H), and ligand binding domain (LBD). The NTD is composed of about 550 amino acids, which is the primary effector region and responsible for transactivation. The DBD is about 70 amino acids mainly responsible for recognizing binding sites on ARE (androgen responsive element). The hinge region connects the DBD and the LBD, which contains the ligand-dependent nuclear localization signal sequence. The LBD interacts with androgen, steroidal or nonsteroidal agonists or antagonists. K845 (Lys 845) and K847 (Lys847) are the two conserved ubiquitination sites on AR, and K847 is the primary ubiquitination site for Skp2-mediated AR degradation. **B.** Differentiated functions of E3 ubiquitin ligases on AR. AR is regulated by a number of ubiquitin E3 ligases for either activity enhancement or protein degradation. RNF6 and Siah2 are AR E3 ubiquitin ligases that promote AR transcription activity by ubiquitination, while MDM2 and CHIP are AR E3 ligases that induce ubiquitin-mediated AR degradation. Skp2 is recently reported as an E3 ubiquitin ligase for AR degradation.

domains of AR protein: N-terminal (NTD), DNA binding (DBD), hinge (H), and ligand binding (LBD), each of which has its distinct function (Fig.1A). A ligand-dependent nuclear localization sequence (NLS) spans both DBD and LBD of AR, and functions to assist the import of androgen-AR complex into nucleus of cells. The function of AR is normally sequestered and inactivated by Hsp90 in cytoplasm. Upon the binding of androgen, the activation of AR is initiated with a consequent conformational change, which dissociates Hsp90 from AR to release the inhibition imposed by Hsp90. Sequentially, androgen-AR complex is shuttled to nucleus with the assistance of NLS. Once in nucleus, the androgen-AR forms homodimers through intra- and inter-molecular N/C interaction of AR, and then binds with the androgen response element (ARE) on the DNA sequence of androgen responsive genes [8]. After recruiting necessary cofactors, androgen-AR complex is able to modulate the activities of these genes by either turning them on or off. Some coregulators including hRad9 and cyclin D1 can inhibit the N/C interaction of AR to modulate the AR transcription activity [9]. Recently, p14ARF was reported

to attenuate AR activity by perturbing the N/C interaction through its binding with N and C termini of AR [10]. So far, more than 1785 human genes are reported to be the androgen responsive genes or to contain androgen responsive elements, which are regulated by androgen at the expression level [6]. For example, prostate-specific antigen (PSA) is the prototype of androgen responsive gene in prostatic epithelial cells.

### Androgen deprivation therapy (ADT) in clinical practice

The morbidity and mortality of PCa are still significantly higher than other human cancers in American men [4], and it remains a big challenge to improve the clinical outcome with the application of effective medical interventions. In majority of cases, the diagnosis of PCa is frequently reported in aged male population, indicating that the malignancy progression is slow and insidious. However, in some cases, PCa progresses in an aggressive and virulent style post diagnosis, which permits very few effective medical options to control the disease. As a result, the treatments are mainly palliative. Therefore, it

is necessary and essential to have the early detection and diagnosis of PCa, which can allow clinicians to treat PCa patients successfully with more options. The medical interventions for PCa at the early stage include the surgical removal of localized tumors containing cancerous lesions, radiotherapeutic approach to eradicate cancerous cells, cryosurgical approach, or just simply close surveillance without any treatment. For PCa at the late stage with distant metastasis, the most commonly applied approach is the androgen deprivation therapy (ADT) that is based on targeting androgen-AR signaling. ADT application is accepted by clinicians as an effective chemotherapeutic approach on PCa because it not only shrinks the size of primary tumors but also restrains the growth of metastatic cancer cells [2,11-14]. More impressively, after the initiation of the therapy, ADT rapidly reduces PCa symptoms, such as severe pain that patients suffered from bone metastasis. However, such an effective inhibition on malignancy normally comes to an end in an average period of 12 to 33 months after therapy, and many patients suffer the relapse of PCa resulted from the recurrent growth of cancer cells. In addition, cancers resistant to ADT grow in a more aggressive and virulent manner, resulting in a severer and wider spread of metastasis, and eventually causes deaths [15, 16]. The recurrent growth of PCa, often referred as castration resistant prostate cancer (CRPC), is clinically very difficult to control due to the drug resistance [3]. In spite of all these clinic advantages, ADT is somehow controversial in terms of clinical indications and options for different approaches. ADT is indicated by FDA in the US only for the palliation of symptomatic metastases and neoadjuvant therapy. Common approaches for ADT include the application of androgen antagonists or analogs with similar functions. ADT application often causes serious side effects and unnecessary symptoms associated with hormone deficiency in PCa patients, such as loss of libido, erection dysfunction, and hot flashes. In addition, ADT may also lead to more serious condition, such as insulin resistance and diabetes, coronary heart disease, osteoporosis and pathologic fractures. Therefore, an optimal management of ADT in PCa patients may apply only after these requirements above are fully evaluated: a), the stages and symptoms indicated for ADT; b), the approach and treatment regimens individually tailored for patients; and c), the strategies to address and manage adverse side effects after therapy.

### **Regulation of androgen receptor by E3 ubiquitin ligases**

Dysregulation of AR signaling pathway, most likely through AR, is associated with the formation and development of primary PCa as well as CRPC progression. ADT is meant to impede the interaction of androgen

and AR to nullify the potent driver of the carcinogenesis of the prostate. However, PCa cells gain abilities to continue their proliferation in an environment with a minimum activation of AR signaling pathway. Current theories state PCa cells still depend on AR for their growth under androgen-depletion environments, therefore AR as a unique oncogenic factor in PCa receives particular attentions in research and clinic. Studies on the AR signaling have shed light on molecular mechanisms of pathological phenotypes, which will provide valuable information leading to the cure of PCa. A large body of evidence reveals that the activities and levels of AR are regulated by many cofactors at transcriptional and posttranslational levels. Posttranslational modifications of proteins, referring ubiquitination, sumoylation, methylation, acetylation and phosphorylation, are essential biological processes in maintaining the inner homeostasis of cells through mediating protein expression and activity. Ubiquitination, for instance, has been extensively studied in the past two decades since its discovery in early 80s [17-20]. Ubiquitin was first identified in 1975 as an unknown property of protein expressed in all eukaryotic cells [21-23]. A few years later, a group of scientists from the US and Israel successfully revealed the mechanism that involves the ubiquitin-ubiquitination system. Ubiquitination procedure executes its function in three steps: a) E1 of ubiquitin-activation, b) E2 of ubiquitin-conjugation, and c) E3 of ubiquitin-ligation. A number of E3 ligases have been discovered since then, some of which are involved in AR regulation by protein degradation or activity promotion. RNF6, for instance, was identified to be an ubiquitin E3 ligase for AR protein through proteomic screening, and induces AR ubiquitination to increase AR transcriptional activity [24]. By contrast, MDM2 (mouse double minute 2 homolog), an E3 ubiquitin ligase, functions to degrade AR through ubiquitination process either in a phosphorylation-dependent or -independent pattern. Lin *et al.* showed that AR is regulated by MDM2 through ubiquitination and proteolysis [25], and AR modification by MDM2-mediated ubiquitination destabilizes AR proteins and attenuates AR transactivity. In addition, the AR degradation by MDM2 was also reported through a phosphorylation-dependent mechanism. Liu *et al.* found that PAK6 (p21 activated kinase 6) inhibits PCa growth by phosphorylation of AR and MDM2 [26]. PAK6 phosphorylates AR at Ser-578 to promote the association between AR and MDM2, whereby to activate the MDM2-mediated proteasomal degradation of AR. Similarly, AKT (protein kinase B) degrades AR through the phosphorylation-dependent ubiquitination process mediated by MDM2 E3 ligase [25], and MDM2 phosphorylation by AKT increases ubiquitination and degradation of AR protein. CHIP (C-terminus of Hsp70-interacting protein) was reported to degrade unfold AR protein [27]. Recently, Siah2 was reported to be an E3

ubiquitin ligase that contributes to CRPC by regulating AR transcriptional activity<sup>[28]</sup>. The study revealed that Siah2 specifically targets an inactive form of AR protein which is bound to NCOR1 for ubiquitination and degradation, thus to promote the expression of the selected AR target genes. Furthermore, Siah2 is required for PCa growth under an androgen deprivation condition. Therefore, a number of studies have revealed that various E3 ubiquitin ligases are playing differential roles on AR regulation in PCa in given oncogenic environments. Besides these E3 ligases specific for AR aforementioned above, several other E3 ligases, such as E6-AP (E6-associated protein) and TRIM68, also regulate the ubiquitination of AR co-factors<sup>[27, 29, 30]</sup>. Most recently, SKP2 has been identified as an AR-specific E3 ubiquitin ligase<sup>[31]</sup>, underscoring the complexity of AR regulation in PCa (Fig. 1).

### **SKP2, a novel E3 ubiquitin ligase for AR degradation**

SKP2 stands for S phase kinase-associated protein 2, and belongs to the multi-protein E3 ubiquitin ligase SCF protein complex that is composed of Skp1, cullin1, Rbox1, and F-box. SKP2 has 424 residues with the feature of F-box protein, and the F-box domain of 47 amino acids is located at the N-terminal region of 94-140 position and the ten leucine-rich repeats (LRR) span from the position 151 to 401. The F-box domain on SKP2 acts as a substrate-specific factor, while LRR assists the interactions between proteins. The role of SKP2 as an E3 ligase is well exemplified by its regulatory effect on p21, a key cell cycle regulator, which was the first report that recognizes SKP2 has an E3 ubiquitin ligase function<sup>[32]</sup>. SKP2 contributes to many cell cycle related factors, such as CDK2, CKS1B, and p27<sup>[33-36]</sup>. The recent discovery about SKP2 directly targeting AR for degradation, together with its regulation on estrogen receptor<sup>[37]</sup>, underscores the novel function of SKP2 on nuclear factors in cancers<sup>[31]</sup>. In order to elucidate that SKP2 is an E3 ubiquitin ligase for AR, authors demonstrated that AR protein level was decreased by SKP2 overexpression but was increased by SKP2 knockdown via regulation of ubiquitin-mediated proteasomal degradation. Remarkably, AR protein levels in two AR negative PCa cell lines, PC3 and DU145, were restored to a detectable level upon SKP2 knockdown. Mechanistically, this study decoded a novel relationship of SKP2 and AR in PCa cells in 2 ways. 1), SKP2 recognizes AR at a unique ubiquitination site. Authors proposed that two conserved ubiquitination sites on AR, K845 and K847, were candidate lysine residues for SKP2-mediated AR degradation. The results demonstrated that AR ubiquitination was greatly diminished in K847A mutant, while K845A mutation did not affect AR ubiquitination level. Thus, SKP2 acts as AR-specific E3 ligase that ubiquitinates and degrades AR via the ubiquitination site K847 on AR. As RNF6 enhances AR transcriptional

activity through ubiquitination at K845<sup>[24]</sup>, these studies on K845 and K847 suggest that AR ubiquitination is distinctly orchestrated by SKP2 and RNF6 in PCa cells. 2), AR ubiquitination by SKP2 is associated with PTEN/PI3K network. In addition to functioning as an E3 ligase for multiple factors, SKP2 is also a proto-oncogene in various cancers including PCa. Aberrant regulation of SKP2 results in alternations of other essential signaling pathways, which in turn affecting AR expression. PTEN loss upregulates SKP2, and SKP2 enhances AKT activity via ubiquitination<sup>[38]</sup>. On the other hand, AKT protects SKP2 degradation from APC/Cdh1 complex<sup>[39]</sup>, and AKT inhibition upregulates AR expression by disruption of phosphorylation-dependent ubiquitination or activation of receptor tyrosine kinases<sup>[40]</sup>. This study revealed that SKP2 knockdown reduces AR degradation by abrogation of SKP2-mediated ubiquitination, suggesting that AR function may be synergistically controlled by multiple pathways in PCa cells. This finding was further confirmed by results of the study in which BEZ235 (a dual inhibitor of PI3K/mTOR) treatment resulted in suppression of PCa cells by regulation of AKT, SKP2 and AR. BEZ235 treatment reduces the activated AKT (pAKT) and consequently decreases SKP2 level, resulting in the upregulation of AR. However, Akt inhibition can also decrease AR level<sup>[41]</sup>, which may explain the reduced level of AR when cells are treated with a high dose of BEZ235. Collectively, SKP2-mediated AR ubiquitination is a novel mechanism on AR regulation, which crosstalks with PTEN/PI3K signaling in PCa.

### **Future directions in research**

AR signaling pathway is essential for the initiation, progression and metastasis of PCa, and emerging evidence has shown that the growth of CRPC is still dependent on AR function after ADT<sup>[42, 43]</sup>. In CRPC, AR activity is abnormally elevated by adapting AR and co-factors to be a more sensitive status under a trace amount of androgen microenvironment, or by structural modifications that enables AR to be responsive to non-androgen steroid hormones. Given the oncogenic impact of AR signaling in PCa, the management of AR activity should be exceptionally important and rewarding to control PCa successfully at bedside. Yet, lacking of a complete understanding of the mechanisms equips us limited avenues for CRPC patients. Even for these well-documented oncogenic pathways, the crosstalk among them remains unclear, especially from the pharmacological perspective. The report on SKP2-mediated AR ubiquitination, together with other mechanisms on AR regulation, truly provides us with deep insights into understanding the role of AR in PCa. In addition, this study may reveal a clue or explanation on the decreased levels of AR in some cells of PCa specimens,

and the relevance of SKP2-AR mechanism on malignancy *in vivo* is worthy of further investigation.

### Conflicting interests

The authors have declared that no competing interests exist.

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