REVIEW

Androgen receptor mutations for precision medicine in prostate cancer

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Abstract

Hormonal therapies including androgen deprivation therapy and androgen receptor (AR) pathway inhibitors such as abiraterone and enzalutamide have been widely used to treat advanced prostate cancer. However, treatment resistance emerges after hormonal manipulation in most prostate cancers, and it is attributable to a number of mechanisms, including AR amplification and overexpression, AR mutations, the expression of constitutively active AR variants, intra-tumor androgen synthesis, and promiscuous AR activation by other factors. Although various AR mutations have been reported in prostate cancer, specific AR mutations (L702H, W742L/C, H875Y, F877L, and T878A/S) were frequently identified after treatment resistance emerged. Intriguingly, these hot spot mutations were also revealed to change the binding affinity of ligands including steroids and antiandrogens and potentially result in altered responses to AR pathway inhibitors. Currently, precision medicine utilizing genetic and genomic data to choose suitable treatment for the patient is becoming to play an increasingly important role in clinical practice for prostate cancer management. Since clinical data between AR mutations and the efficacy of AR pathway inhibitors are accumulating, monitoring the AR mutation status is a promising approach for providing precision medicine in prostate cancer, which would be implemented through the development of clinically available testing modalities for AR mutations using liquid biopsy. However, there are few reviews on clinical significance of AR hot spot mutations in prostate cancer. Then, this review summarized the clinical landscape of AR mutations and discussed their potential implication for clinical utilization.

Introduction

Prostate cancer is one of the most frequently diagnosed cancers in developed countries. Characteristically, prostate cancer depends on androgen receptor (AR) signaling for its carcinogenesis, development, and progression (Basu & Tindall 2010). Meanwhile, androgen deprivation therapy has been a standard treatment for metastatic hormone-sensitive prostate cancer (mHSPC) (Shiota & Eto 2016). However, most prostate cancers eventually progress to castration-resistant prostate cancer (CRPC). Recently, novel AR pathway inhibitors (ARPIs) such as the cytochrome P17 (CYP17) inhibitor abiraterone acetate and second-generation antiandrogens such as enzalutamide,
apalutamide, and darolutamide have proven to prolong survival in patients with mHSPC and CRPC (Shiota & Eto 2016, Harada et al. 2021).

Prostate cancer can acquire resistance to hormonal treatments including surgical or medical castration (leuprolrelin, goserelin, and degarelix), antiandrogens (bicalutamide, flutamide, enzalutamide, apalutamide, and darolutamide), CYP17 inhibitors (abiraterone), and several steroidal agents (estrogen and glucocorticoid), and aberrant activation of AR signaling plays a crucial role in this process (Shiota et al. 2011a,b, 2012). Aberrant activation of the AR signaling pathway in prostate cancer has been attributed to a number of mechanisms, including AR amplification and overexpression, AR mutations, expression of constitutively active AR variants, intra-tumor androgen synthesis, and promiscuous AR activation caused by other factors (Shiota et al. 2011a,b, 2012).

Among them, 10–20% of CRPCs are known to possess somatic AR gene mutations (Taplin et al. 2003). Actually, recent studies on whole genome sequencing by next-generation sequencing (NGS) in CRPC have revealed mutational landscape of prostate cancer and showed that AR gene mutations are one of most frequently observed mutated genes (GRYsO et al. 2012). Interestingly, mutant AR can promiscuously be activated by adrenal androgens, non-steroidal androgens, and even antiandrogens (Waltering et al. 2012). In addition, clinical data between AR mutations and the efficacy of ARPs have recently been accumulating (Huang et al. 2022). Thus, the clinical importance of AR mutations has been suggested, and information about the mutation status could contribute to the selection of effective treatment for individual patients. Currently, precision medicine utilizing genetic and genomic data is becoming to play an increasingly important role to choose suitable treatment for the patient in clinical practice for prostate cancer (Malik et al. 2019). However, there are few reviews on clinical significance of AR mutations in prostate cancer. In this review, we summarized the clinical landscape of AR mutations and discussed their potential implication for clinical utilization.

Research history on AR mutation in prostate cancer

In 1990, Veldscholte et al. at Erasmus University discovered a point mutation (T868A in codon 910 of AR cDNA; this mutation is equivalent to T878A in codon 920 of AR cDNA based on the human reference genome Hg19 and codon numbering was based on the human reference genome Hg19 thereafter) in the ligand-binding domain (LBD) of the AR gene in LNCaP prostate cancer cells and reported that this AR mutant was activated in vitro by androgens as well as progesterone, estrogen, and the antiandrogen cyproterone acetate (Table 1) (Veldscholte et al. 1990). Subsequently, AR mutations such as H875Y and L702H/T878A were found in CWR22 and MDA PCa 2a prostate cancer cell lines, respectively (Tan et al. 1997, Zhao et al. 1999).

In 1992, Newmark et al. at Johns Hopkins University reported the first case of a somatic mutation in the LBD of AR (V731M) among 26 specimens of untreated organ-confined prostate cancer (Table 1) (Newmark et al. 1992). Subsequently, Culig et al. at University of Innsbruck, Suzuki et al. at Chiba University, and others found somatic mutations in the tumor tissues from patient who showed refractory to endocrine treatment, indicating that AR mutations were associated with disease progression and resistant to endocrine therapy (Table 1) (Culig et al. 1993, Suzuki et al. 1993, Gaddipati et al. 1994, Taplin et al. 1995, 1999, Suzuki et al. 1996, Tilley et al. 1996, Marcelli et al. 2000, Tepper et al. 2002).

In addition to treatment resistance, AR mutations such as T878A and W742C/L were reported to be associated with antiandrogen withdrawal syndrome (AWS), suggesting the clinical importance of AR mutations in treatment navigation (Table 1) (Suzuki et al. 1996, Hara et al. 2003). Meanwhile, in the Cancer and Leukemia Group B Study 9663, AR mutations were detected in 5 of 48 CRPC tumors, but there was no association between AR mutations and antiandrogen withdrawal response or survival (Table 1) (Taplin et al. 2003). This null result may be because all AR mutations were analyzed together even though each AR mutation may have a different clinical impact, suggesting the importance of evaluating AR mutations individually. In addition, promiscuous activation of mutated AR by steroids and antiandrogens were shown to contribute cancer cell growth non-cognate ligand (Table 1) (Zhao et al. 2000). Thus, it was demonstrated that AR mutations were closely associated with tumor response to endocrine therapy in prostate cancer.

In the 2000s, NGS was developed, and it allowed the sequences of entire genomes or targeted regions of DNA or RNA to be determined. After the discoveries of AR mutations using traditional techniques such as Sanger sequencing, various studies were performed by NGS using tissues and blood from patients, and they revealed the landscape of gene mutations, in which AR mutations were detected reproducibly (Tolkach & Kristiansen 2019).
Among them, several mutations in the LBD were frequently and reproducibly detected in tissues and circulating tumor DNA (ctDNA) from patients with prostate cancer by NGS (Table 2). In a study using prostate cancer tissues, T878A (2.5%), L702H (2.3%), W742C (2.3%), H875Y (2.0%), W742L (0.5%), T878S (0.5%), and F877L (0.2%) were detected in 444 tumor tissues from 429 patients with metastatic CRPC (mCRPC) (Abida et al. 2019). Similar detection rates of AR mutations were reported in studies using ctDNA from patients with prostate cancer (Table 2). Among these studies, Ledet et al. performed the largest investigation of AR mutations using Guardant360 among 892 patients with advanced prostate cancer and detected L702H (24.6%), T878A (14.5%), H875Y (11.4%), W742C (8.4%), W742L (3.8%), F877L (2.1%), and T878S (1.6%) (Ledet et al. 2020). Consistently, a meta-analysis revealed that L702H (3.4%), H875Y (4.9%), and T878A (4.4%) were the most prevalent mutations across 1614 patients with CRPC from 21 studies (Snaterse et al. 2022). Similarly, recurrent mutations on these sites (L702H in T75 samples,
<table>
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<td>892</td>
<td>Advanced prostate cancer (predominantly CRPC)</td>
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<td>NGS (Gaurdian360)</td>
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</table>

Table 2: Frequency of driver androgen receptor mutations in the ligand-binding domain.

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<td>1 (0.1%)</td>
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<td>10 (2.0%)</td>
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AR, androgen receptor; CRPC, castration-resistant prostate cancer; ctDNA, circulating tumor DNA, ddPCR, droplet digital PCR; NA, not available; NGS, next-generation sequencer.
W742C/L in 36 samples, H875Y in 45 samples, F877L in 5 samples, and T878A/S in 61 samples) were reported from 9087 patients/9377 samples in 23 studies, which was retrieved from cBioPortal (http://www.cbioportal.org/) (Fig. 1). In addition to recurrent mutations in hot spot residues, interestingly, other missense substitution in hot spot residues such as W742F, W742R, and T878G were reported (Armenia et al. 2018, Borgmann et al. 2018, Dong et al. 2021a). Notably, each hot spot AR mutation was observed commonly among ethnics (Table 2).

**Changes of binding affinity attributable to AR mutation**

Missense mutations in the LBD of AR can alter the binding affinity of ligands and result in activation by ligands other than testosterone and dihydrotestosterone (DHT). As indicated in Fig. 2A, the residues of hot spot mutation are located close to the binding site of the cognate ligand testosterone (Lallous et al. 2016). Lallous et al. determined the in vitro effects of four antiandrogens (enzalutamide, hydroxylutamide, bicalutamide, and apalutamide) and four steroids (DHT, progesterone, estradiol, and hydrocortisone) on AR variants with mutations in the LBD (Lallous et al. 2016). Intriguingly, as presented in Table 3, several mutants responded differentially to antiandrogens and steroids (Lallous et al. 2016). In addition, recent studies demonstrated an antagonistic effect of darolutamide on AR mutants (Moilanen et al. 2015, Sugawara et al. 2019, Lallous et al. 2021). Because abiraterone is administered with prednisone and it blocks the catalysis of progesterone into androstenedione, abiraterone may result in increased levels of glucocorticoid and progesterone (Attard et al. 2008). Therefore, the alterations of steroid levels following abiraterone treatment may affect tumor response through aberrant AR signaling caused by AR mutation.

Among the residues of hot spot mutation, L702 and W742 are located close to the binding site of testosterone, which may result in an altered activation by DHT (Table 3). As well, W742 and T878 are located close to the binding site of bicalutamide, and then mutations in these sites may lead to an altered function of bicalutamide from antagonist to partial agonist or agonist (Fig. 2B and Table 3). Similarly, H875, F877, and T878 are located close to the binding site of enzalutamide, and then mutations in these sites may lead to an altered function of enzalutamide from antagonist to partial agonist or agonist (Fig. 2C and Table 3). Interestingly, W742 is located close to the binding site of bicalutamide, but not enzalutamide, and then bicalutamide becomes agonistic to W742L/C mutations while enzalutamide remains antagonistic (Fig. 2B, C and Table 3). Similarly, F877 is located close to the binding site of enzalutamide, but not bicalutamide, and then enzalutamide becomes partial agonistic to F877L mutation while bicalutamide remains antagonistic (Fig. 2B, C and Table 3). In addition,
W742L mutation was shown to change its formation into agonistic by bicalutamide but remains antagonistic by darolutamide (Sugawara et al. 2019). Thus, the structures of ligands such as steroids and antiandrogens is critical to their function to mutated AR. Actually, since enzalutamide and apalutamide have very similar molecular structure, they show similar pharmacological profiles to mutated AR (Table 3) (Tran et al. 2009, Clegg et al. 2012).

Clinical implications of AR mutation for precision medicine

Through altered ligand affinity, AR mutations of the LBD are believed to affect the clinical outcome of hormonal therapy. As presented in Table 4, the clinical implications of AR mutations regarding the effects of several hormonal treatments were estimated from in vitro data on antiandrogens and steroids (presented in Table 3). However, because AR L702H and W742L/C mutations were less sensitive to DHT, prostate cancer cells with these AR mutations may have intrinsic non-dependency on AR (Table 4).

Abiraterone

Several AR mutations including L702H, T878A, and T878S were demonstrated to emerge in ctDNA after treatment with abiraterone (Romanel et al. 2015). Consistently, detection of the AR L702H or T878A mutation in ctDNA before treatment was associated with poor responses to abiraterone, with zero of four patients exhibiting decreases of prostate-specific antigen (PSA) levels (Romanel et al. 2015). Similarly, another study demonstrated that only one of eight post-docetaxel patients carrying the AR L702H or T878A mutation experienced PSA decline during abiraterone treatment (Conteduca et al. 2017). Similarly, the H875Y mutation was reported to emerge after progression on abiraterone treatment (Azad et al. 2015). Consistently, the H875Y and T878A mutations were shown to be activated by progesterone in an experimental model (Hou et al. 2022). In contrary, two patients carrying the H875Y mutation without AR amplification displayed excellent PSA responses to abiraterone, raising controversy regarding the clinical significance of the H875Y mutation in abiraterone treatment and then requiring further investigation (Torquato et al. 2019). Thus, AR L702H,
T878A, and T878S are clinically associated with abiraterone resistance, consistent with the in vitro data (Table 4).

**Enzalutamide and apalutamide**

Similarly, several AR mutations including H875Y and T878A emerged in ctDNA after treatment with enzalutamide (Romanel et al. 2015). Consistently, AR mutation (H875Y or T878A) was associated with a short duration of response to enzalutamide and an increased fraction of AR mutants during enzalutamide treatment (Wyatt et al. 2016). Meanwhile, the fraction change of AR mutants (L702H, W742L, or W742C) during enzalutamide treatment varied among patients, suggesting the AR L702H and W742L/C mutations did not reflect enzalutamide resistance (Wyatt et al. 2016). Intriguingly, enzalutamide and apalutamide are antagonistic to AR W742L/C mutants, but they had less potent suppressive effects on the activation of AR W742L/C mutants by androgen than darolutamide, suggesting darolutamide may be the preferred agent for patients carrying AR W742L/C mutations (Sugawara et al. 2019). In addition, AR F877L is another mutation associated with enzalutamide resistance. First, the AR F877L mutation was found to emerge after enzalutamide resistance in LNCaP prostate cancer cells (Joseph et al. 2013, Korpal et al. 2013). However, this mutation was rarely (0–2%) found among patients with CRPC (Table 2). Wyatt et al. detected the AR F877L mutation in only one patient after progression during enzalutamide treatment (Wyatt et al. 2016). Thus, AR H875Y, F877L, and T878A are clinically associated with enzalutamide resistance, consistent with the in vitro data (Table 4). However, darolutamide was shown to be more effective to these mutants than enzalutamide in an experimental model (Borgmann et al. 2018).

**Glucocorticoid**

The phase II SWITCH study indicated that switching from prednisone to dexamethasone during treatment with abiraterone resulted in PSA and radiographic responses in some patients with mCRPC (Romero-Laorden et al. 2018). Interestingly, this study evaluated AR copy number alteration and AR mutations including L702H and T878A in ctDNA, and the best treatment effect was observed in patients with the AR T878A mutation who received abiraterone plus dexamethasone. Conversely, poor responses were noted patients with AR amplification (Romero-Laorden et al. 2018). Consistently, a patient with multiple AR mutations (L702H and T878A) displayed PSA decline after abiraterone plus dexamethasone treatment,
but no response to abiraterone plus prednisone was noted (Torquato et al. 2019). Because the AR T878A mutant is activated by progesterone and this effect is enhanced by abiraterone but suppressed by continuous low-dose dexamethasone, abiraterone plus dexamethasone may be effective against mutated AR (Attard et al. 2012).

Intriguingly, the AR L702H mutant was stimulated by cortisol and prednisolone but not by dexamethasone, whereas the AR T878A mutant was not stimulated by cortisol, prednisolone, and dexamethasone in an in vitro assay (Snaterse et al. 2022). Thus, patients with AR mutations (L702H or T878A) may benefit from switching the steroid from prednisone/prednisolone to dexamethasone.

### Estrogen

In addition, the H875Y mutation was indicated to be sensitive to estradiol in in vitro experiments (Lallous et al. 2016). In a case report, a patient carrying the H875Y mutation experienced rapid PSA elevation after treatment with the estrogen agent diethylstilbestrol (Vasudevamurthy et al. 2017). Therefore, the administration of estrogens should be avoided in patients carrying the H875Y mutation.

### AWS

Furthermore, AWS may be observed after antiandrogen withdrawal if antiandrogens are agonistic to the AR mutation. After the seminal discovery by Suzuki et al. of chlorormadinone acetate withdrawal syndrome in patients carrying the T878A mutation, several studies described the association between AR mutation and AWS (Suzuki et al. 1996, Lorente et al. 2015, Leone et al. 2018). The agonistic effects of flutamide on the H875Y or T878S mutant and those of bicalutamide on the W742L or W742C mutant were demonstrated by in vitro and in vivo experiments, suggesting these mutations may cause AWS after the withdrawal of each antiandrogen (Fenton et al. 1997, Taplin et al. 1999, Hara et al. 2003, Terada et al. 2010). Meanwhile, ASW after the withdrawal of enzalutamide and abiraterone has been reported at a frequency of 0–5%, and this finding may also be attributable to the agonistic effect of each agent on AR mutants (Leone et al. 2018). Therefore, information on AR mutations may be helpful for predicting the possibility of AWS in addition to the effectiveness of ARPs.

Taken together, the clinical relevance of AR mutants including L702H, W742L, W742C, H875Y, F877L, T878A, and T878S has been demonstrated, as cBioPortal currently defines these mutants as driver missense mutations. However, since high-level evidence on clinical implications

<table>
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<tr>
<th>AR mutations</th>
<th>Bioavailability</th>
<th>Enzalutamide</th>
<th>Apalutamide</th>
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<tr>
<td>L702H</td>
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<td>W742L/C</td>
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<td>H875Y</td>
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<td>T878A/S</td>
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**Table 4** Expected response to androgen receptor pathway inhibitors by driver androgen receptor mutations.

- R, resistance; S, sensitive.
- Bold indicates clinically supported data.
- Possible intrinsic resistance due to low-dependency on DHT.

AWS in addition to the effectiveness of ARPIs.
of AR mutation lacks currently, further investigations would be required in the future.

**Spatiotemporal alterations of AR mutations in prostate cancer**

In treatment-naïve prostate cancer, almost no AR mutation was detected in tissues and ctDNA (Cancer Genome Atlas Research Network 2015, Vandekerkhove et al. 2019). Meanwhile, comprehensive analyses in prostate cancer tissues robustly revealed increased genomic alterations including AR mutations after the progression to CRPC (Grasso et al. 2012, Beltran et al. 2013, Robinson et al. 2015, Mateo et al. 2020). Similarly, in the phase II PROPHECY trial, de novo AR somatic mutations were detected in 8 of 49 patients after abiraterone treatment (Gupta et al. 2021). In other phase II trials, the frequency of AR mutations detected in ctDNA was reported to change after abiraterone or enzalutamide exposure (Annala et al. 2021, McKay et al. 2021). Thus, AR mutations were detected more frequently after the emergence of resistance to hormonal therapy, but AR mutants vulnerable to treatment may regress in post-treatment samples.

In addition to temporal changes of AR mutations, spatial intra-tumor heterogeneity has been reported (Boutros et al. 2015, Su et al. 2018, VanderWeele et al. 2019). AR aberrations occurred on the off-trunk, indicating that AR genomic changes occur after tumorigenesis and vary spatially in an intra-patient manner, potentially leading to spatial heterogeneity in patients (Gundem et al. 2015). In fact, AR mutations were reported to be extremely rare in lung metastasis without liver involvement compared to the incidence of bone-only or liver metastasis, suggesting that the status of AR mutation appears to differ among tumor sites (Gong et al. 2021).

**Clinical development of tests for detecting AR mutations**

To date, various mechanisms other than AR mutations, including AR gene amplification, variant forms of AR, and non-AR pathways, have been described to induce resistance to hormonal treatments including novel ARPIs in prostate cancer (Shiota et al. 2012). Therefore, comprehensive assessment of the aberrations leading to treatment resistance is necessary for the accurate prediction of treatment response. Currently, clinical comprehensive assessments of genomic alterations can be performed via cancer genome profiling using NGS-based assays for target gene regions such as Foundation CDx, Foundation Liquid, and MSK-IMPACT. However, cancer genome profiling is expensive and time-consuming. Thus, its use is limited clinically, and performing multiple tests in individual patients is economically unfeasible.

Clinical information on the AR mutation status in addition to the AR copy number and AR variant status are critically important for selecting effective treatments for individual patients. Actually, recent analysis by liquid biopsy demonstrated that co-occurrence of AR mutation and amplification was observed in 5 (33.3%) among 15 clinical CRPC patients with AR mutation, in addition to co-occurrence with other genomic alterations (Kohli et al. 2020, Dong et al. 2021b). As well, AR mutation in addition to APC and KIT mutations was shown to increase in mCRPC compared to mHSPC (Kohli et al. 2020). Because the AR mutation status and other alterations thus change throughout the clinical course, it is necessary to monitor AR alterations during the clinical course. Another clinical issue in detecting AR mutations is that obtaining tissues for sequencing is often difficult because of the invasiveness of the procedures and limited access to suitable tissues, especially when tissues are needed sequentially after treatment. In addition, spatial heterogeneity leads to difficulty in capturing all genomic alterations in the host. Therefore, liquid biopsy is a promising approach for monitoring the AR mutation status, and it is more feasible and comprehensive than tissue biopsy. In fact, Wyatt et al. demonstrated the high concordance between metastatic tissue and ctDNA and high detection of AR mutations in ctDNA. In particular, six AR mutations were detected in both metastatic tissue and ctDNA, whereas one AR mutation was detected in ctDNA but not in metastatic tissue (Wyatt et al. 2017). However, the amount of samples for analysis via liquid biopsy is limited. Therefore, multiplex assay or NGS for AR mutations using various technologies that enable the detection of multiple AR mutations simultaneously needs to be developed for clinical application. Droplet digital PCR technology has the advantages of lower costs and a better limit of detection than conventional NGS. Therefore, technologies based on droplet digital PCR may be more suitable for clinical application to permit multiple testing with high sensitivity for monitoring the AR mutation status.

**Conclusion**

Currently, clinical findings on the association between AR mutations and treatment response to various ARPIs...
in prostate cancer are limited as most of the studies cited in this review investigated low numbers of patients, and continuous accumulation of knowledge in this field would lead to improved prediction of treatment responses to hormonal manipulation associated with the AR mutation status. Monitoring AR mutation through the clinical course of advanced prostate cancer is clinically important for ensuring the accuracy of the selection and timing of treatments for individual patients. At present, there is no clinically available test for detecting AR mutations excluding cancer genome profiling. Thus, clinically available tests for AR mutation represent an unmet need in advanced prostate cancer treatment.

Declaration of interest
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