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Treatment-induced changes in the Androgen receptor axis: liquid biopsies as diagnostic/prognostic tools for prostate cancer

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Abstract

Prostate cancer progression and treatment relapse is associated with changes in the androgen receptor axis, and analysis of alternations of androgen receptor signaling is valuable for prognostics and treatment optimization. The profile of androgen receptor axis is currently obtained from biopsy specimens, which are not always easy to obtain. Moreover, the information acquired only provides a snapshot of the tumor biology, with strict spatial and temporal limitations. On the other hand, circulation is easily accessible source of both circulating tumor cells and circulating tumor DNA, which can be sampled at numerous time points. This Review will explore the potential use of androgen receptor axis alternations detectable in the blood in therapeutic decision-making and precision medicine for advancing metastatic castration-resistant prostate cancer.

1. Introduction

Despite the high survival rates and recent progress in treatment modalities, prostate cancer (PCa) remains an important healthcare issue with a widespread socio-economic impact (Ferlay et al., 2015). In the majority of cases, PCa is organ-confined at the moment of diagnosis and a cure can be provided by prostatectomy or radiation therapy (>90% progression free survival). However, the more advanced forms of the disease can metastasize, mainly to the bone (90%) or lung (46%) (A. J. Chang, Autio, Roach III, & Scher, 2014) and are incurable.

The benchmark treatment for metastatic PCa is androgen deprivation therapy (ADT) combined or not with chemotherapy. Unfortunately, in most cases the disease will become resistant to ADT, hence the classification of “castration-resistant prostate cancer” (CRPC). At this stage, hormone therapy with AR-targeting drugs is considered. Unfortunately, although at first these approaches can be effective, the development of resistance is inevitable in most cases (Hotte & Saad, 2010). Development of resistance happens mostly via the reactivation of the AR axis, thus several novel compounds targeting the AR axis have been developed (Andersen et al., 2010; Helsen et al., 2014; Njar & Brodie, 2015).

During the progression of metastatic castration resistant prostate cancer (mCRPC), serum PSA measurements and bone scans may provide valuable prognostic information (Cornford et al., 2016), but because they do not report on the underlying mechanisms, their use in the development of personalized precision therapy is limited. To have an idea on the mechanisms that are involved in cancer progression

for each individual patient, one would need markers that provide more precise information on the relevant metastases. Unfortunately, sampling multiple metastases or identification of the most relevant metastases is nearly impossible in current clinical practice.

One alternative source of information on disease progression could come from liquid biopsies. The term 'liquid biopsies' can indicate urine, blood, saliva or even spinal fluids. These can serve as minimally invasive diagnostic sources of information because biomolecules originating from tumor masses leak into the circulation. Like for most cancers, for prostate cancer, blood would be the preferred liquid biopsy because of the ease of collection and reproducible processing modalities. While blood has traditionally been used to follow PSA and other protein and metabolic markers, more recently, it is used as a source of tumor lipids (Butler, Centenera, & Swinnen, 2016), RNA (Sita-Lumsden, Dart, Waxman, & Bevan, 2013), DNA (Wyatt et al., 2016), exosomes (Bobrie & Théry, 2013) and tumor cells (de Bono et al., 2008). In recent years, circulating tumor DNA (ctDNA) and the circulating tumor cells (CTCs) were added as blood-derived markers. Circulating tumor DNA provides robust information on irreversible changes of the genome in the tumor cells it originates from. CTCs on the other hand can be interrogated not only at the level of their DNA, but also at the level of other biomolecules or signaling pathways. Currently, CTC enumeration by the use of CELLSEARCH[®] is approved by Food and Drug Administration (FDA) as a means of predicting overall survival in metastatic PCa (de Bono et al., 2008). One caveat here is that it is still not clear which of the molecular changes in CTCs reflect the progression of the disease.

Recently, the FDA approved the first blood-based test for the detection of EGFR mutations in non-small cell lung cancer patients (<http://www.fda.gov>) (Voelker, 2016). This allows detection of specific, recurrent mutations, which can help the selection of the patients that could benefit from Erlotinib (Tarceva) therapy.

Because of the central role of the androgen axis in prostate cancer progression and treatment, we will focus our review on the use of ctDNA and CTCs to highlight their potential in therapeutic decision-making and precision medicine for advancing mCRPC.

2. Androgen receptor structure and function

The direct effects of the androgenic hormones like testosterone (T) and dihydrotestosterone (DHT) are mediated by the AR. The *AR* gene is located on chromosome X (q11-12) and contains 8 exons. With a length of 90kb, it is the largest and most complex of all nuclear steroid receptor genes, encoding a protein of 920 amino acids (Lubahn et al., 1988; Tan, Li, Xu, Melcher, & Yong, 2015). The AR is a multi-domain protein composed of the transactivation domain (NTD), a DNA-binding domain (DBD), a hinge region and a C-terminal ligand-binding domain (LBD).

The structure of the AR NTD is believed to be flexible and dependent on its interaction partners (Lavery & McEwan, 2005; McEwan, Lavery, Fischer, & Watt, 2007). The activation function 1 (AF1), which serves as docking station for co-regulators, is located in the NTD (Jenster et al., 1991). This AF1 is composed of two separate but functionally interacting transcription activation units (Tau-1 and Tau-5), each believed to recruit different co-activator complexes (Callewaert, Van Tilborgh, & Claessens, 2006; Van Tilborgh et al., 2013). The FQNFL motif, located upstream of

Tau-1, can interact with the LBD (see below), and is important for the functional interactions between the NTD and the LBD (A. Brinkmann et al., 1999).

The AR DBD recognizes very specific DNA motifs, which occur in promoter or enhancer regions of androgen regulated genes (Denayer, Helsen, Thorrez, Haelens, & Claessens, 2010). This domain consists of two zinc finger modules that are involved in dimerization as well as in positioning of the DNA-recognition α -helix into the major groove of DNA of the response elements (P. L. Shaffer, Jivan, Dollins, Claessens, & Gewirth, 2004).

The LBD of the AR consists of 11 α -helices (H) and 4 β -strands (A. O. Brinkmann et al., 1989; Matias et al., 2000). The ligand binding pocket is buried in the LBD and is surrounded by the N-termini of α -helices 3, 5 and 12. The folding of H12 over the ligand binding pocket forms the core of the activation function 2 (AF2), since this forms a binding groove on the surface of the LBD. This surface is recognized by LxxLL-motifs present on the surface of the co-activators (Estébanez-Perpiñá et al., 2007; Rastinejad, Huang, Chandra, & Khorasanizadeh, 2013; Wurtz et al., 1996) or the FQNLF motif in the NTD (see above). Recently, the dimerization interface of the LBD was reported (Nadal et al., 2017).

The unliganded AR is located in the cytoplasm, in a complex with heat shock proteins (Echeverria & Picard, 2010). When the hormone binds, conformational changes in the LBD occur and the interacting proteins dissociate, thus exposing the nuclear localization sequence (NLS) located in the hinge region between the DBD and LBD. Subsequently, intramolecular N/C interactions occur and the receptor is transported to the nucleus where it forms intermolecular N/C interactions (van Royen, van Cappellen, de Vos, Houtsmuller, & Trapman, 2012). Once in the nucleus, the AR

homodimer regulates gene expression via binding to androgen response elements (ARE), located in the promoter and enhancer regions of the AR target genes. These AREs are organized as inverted repeats of the 5'-AGAACA-3' hexameric consensus sequence (Claessens et al., 2001; Wang & Brown, 2009). Even though the AR directly binds AREs *in vitro*, the *in vivo* accessibility of these binding sites is determined by many factors that shape the chromatin architecture (extensively reviewed in (Arora et al., 2013)). Once bound to the enhancers, the AR N/C interactions are lost and the AR serves as a scaffold for co-regulator proteins and complexes with diverse activities. Some of these proteins/complexes reshape the surrounding chromatin structure by reading, writing, or modifying the histone code, while other complexes mediate the recruitment of general transcription factors to the promoter regions. In this way, the AR and its co-regulators eventually orchestrate the expression of AR target genes (Claessens et al., 2008). The list of AR co-regulators is large and is still expanding (DePriest et al., 2016). The impact of additional levels of control for instance by miRNAs and lncRNAs under normal and pathological conditions are just starting to be unravelled (see contributions by Spahn and Feng elsewhere in this issue).

3. Prostate cancer and resistance to AR targeting treatment

The AR plays a pivotal role in the development, differentiation, homeostasis and secretory function of the normal prostate (Wilson, 2011). The AR is also a key player in many phases of prostate carcinogenesis (Huggins, Stevens, & Hodges, 1941; Scher & Sawyers, 2005). The androgen deprivation therapy (ADT), which is lowering the levels of circulating androgens, is effective for most patients. However, after the initial good response, the disease will progress into what is called mCRPC.

Surprisingly, at this stage of the disease the AR signaling pathway is reactivated, and it can be treated with compounds that block AR signaling either directly as antagonists of the AR protein (e.g. Enzalutamide, ARN-509) or indirectly via blocking androgen biosynthesis (e.g. Abiraterone acetate) (Helsen et al., 2014). These treatments are at first quite effective and have been shown to prolong the overall survival of mCRPC patients (Attard, Reid, et al., 2009; Cabot et al., 2012; De Bono et al., 2011). However, again most patients ultimately progress as the cancer develops resistance towards these drugs.

Multiple molecular mechanisms behind castration or anti-androgen resistance have been proposed (reviewed in (Claessens et al., 2014; Lorente, Mateo, Perez-Lopez, de Bono, & Attard, 2015; Shtivelman, Beer, & Evans, 2014)). Surprisingly, reactivation of the androgen signaling axis explains how PCa cells survive in the absence of androgens or even in the presence of anti-androgens. The most common mechanisms are depicted in Figure 1.

The *AR* gene can be amplified resulting in AR overexpression and persistent signaling (Palmberg et al., 1996). The *AR* gene amplifications and accompanied higher expression levels have been correlated with aggressiveness and therapy response in CRPC (Koivisto et al., 1997; Visakorpi et al., 1995). The concomitant increase in AR protein levels may lead to a sustained signaling under ADT conditions, when circulating androgens concentration is lowered considerably (Cai et al., 2011). Even in the presence of anti-androgens, or androgen synthesis inhibitors, it is well-known that increased AR levels can confer resistance possibly because of partial agonism of the antagonists (Palmberg et al., 1996; Robinson et al., 2015). However, it is also possible that this overexpression produces more splice variants

that lack the LBD (discussed below), thus escaping the control by anti-androgens (Wyatt et al., 2016).

Alternative splicing can result in the truncation of the ligand binding domain, and this will result in a constitutively active transcription factor (Guo & Qiu, 2011). While there is a strong correlation between enhanced AR expression and the detection of splice variants, others have detected genomic events that also can lead to expression of AR truncations (Li et al., 2012). In contrast to cells with genomic AR truncations, cells with aberrant splicing can co-express full size together with AR splice variants. The variants (primarily AR-V7) were first shown to emerge in metastatic PCa after androgen deprivation as well as anti-androgen treatment (Hu et al., 2009). It is likely that cells expressing the splice variants lacking the LBD escape the selective pressure on the AR axis. Clearly such a truncated receptor retains DNA binding and most of its transactivation properties but lost its ligand control (Claessens et al., 2014).

Alternatively, the AR can be mutated, which results in gain of functions rendering the receptor either promiscuous for antagonists or other hormones, or hypersensitive to androgens (Chandrasekar, Yang, Gao, & Evans, 2015). The majority of the mutations found in PCa are missense mutations in the LBD, while NTD and DBD mutations are less frequent (Gottlieb, Beitel, Nadarajah, Paliouras, & Trifiro, 2012; Koochekpour, 2010). Interestingly, one PCa mutation that resides in the LBD dimer interface was proposed to have a stabilizing effect, thus adding another possible mechanism of resistance (Nadal et al., 2017).

Finally, intracrine androgen synthesis/metabolism can be activated thus allowing cells to bypass the low levels of androgens in the circulation by producing androgens

themselves (Cai & Balk, 2011; Rahman, Miyamoto, & Chang, 2004). Moreover, changes in AR co-regulators like the overexpression of steroid receptor coactivators can also be correlated with therapy response (Brooke, Parker, & Bevan, 2008).

4. Circulation as a source of information

Diagnosis and management of PCa has been undoubtedly improved since the introduction of biomarkers (Crawford, Ventii, & Shore, 2014). A biomarker can be any molecule that provides additional information on the disease next to the standard clinical parameters. Such molecules can be detected either in the tumor tissues obtained by biopsy or surgery, or in body fluid samples (Prensner, Rubin, Wei, & Chinnaiyan, 2012). Prostate cancer mainly metastasizes to the bone and lung, and these metastases are not easily biopsied. Fortunately, less invasive means of sampling cancer material through liquid biopsies can still provide a lot of insight into the disease.

Of particular interest are circulating tumor DNA (ctDNA) and circulating tumor cells (CTC). ctDNA can be easily sequentially sampled and analyzed, and hence can be used to follow changes in the tumors during the course of therapy.

Deep sequencing circulating tumor DNA has been applied to mCRPC patients and enabled the detection of mutations and copy number changes that occur before, during or post-treatment (Carreira et al., 2014). Liquid biopsies indeed showed to contain information on the underlying mechanisms of resistance, and thus could lead

to better optimization of therapy. The use of CTCs on the other hand needs more sophisticated sampling and larger blood volumes. While they provide information on a much smaller number of cells, they might provide relevant snap-shots of cancer cell features like the changes in transcriptomes.

We will now discuss the data that indicate the use of plasma or ctDNA and CTCs as source of clinically useful information on the AR signaling axis, which could guide precision therapy in the future.

4.1. *AR* gene copy number gain

In concordance with the observations on biopsies, where amplifications are typically detected in up to 50% of the CRPC cases (Barbieri et al., 2013; Robinson et al., 2015; Visakorpi et al., 1995), *AR* gene gain is detected in 20-60% of plasma DNA or CTCs from mCRPC patients (Leversha et al., 2009; Romanel et al., 2015; D. R. Shaffer et al., 2007).

Recent studies showed that increased *AR* copy number in ctDNA prior to treatment with abiraterone or enzalutamide correlates with poor outcomes (Romanel et al., 2015; Wyatt et al., 2016). Moreover, detection of *AR* gain in pretreatment ctDNA was found to be significantly associated with enzalutamide resistance and shorter progression-free survival and overall survival (Salvi et al., 2016). In addition, the number of previous chemotherapeutic cycles or abiraterone treatment do not affect *AR* copy number (Attard, Swennenhuis, et al., 2009; Carreira et al., 2014; Salvi et al., 2016).

4.2. Treatment-resistance and *AR* point mutations

AR gene point mutations are rarely detected in untreated primary PCa tissue but show selection for in mCRPC, implying that they might be causal for the observed resistance (Grossmann, Huang, & Tindall, 2001; Robinson et al., 2015). Various treatment-induced mutations lead to a switch in the mode of action of anti-androgens to an agonistic mode or a promiscuity of the receptor manifested as increased potential for binding of and activation by other ligands (Grossmann et al., 2001).

AR genomic aberrations are spatially heterogeneous (Gundem et al., 2015) and sequencing of the *AR* coding regions in plasma DNA has shown temporal heterogeneity for *AR* mutations (Azad et al., 2015; Carreira et al., 2014; Lallous et al., 2016; Wyatt et al., 2016). In one particular patient which first relapsed while on Bicalutamide treatment, two mutations (M896V and S889G) were detected. The same patient was then treated with Enzalutamide and after 4 months of treatment, new mutations emerged in the circulation (F877L/T878A and T878A/S889G), while the frequency of single mutations, M896V and S889G drastically dropped (Azad et al., 2015; Lallous et al., 2016).

Another important observation was that some patients with advanced disease had multiple *AR* mutations in their cell free DNA (cfDNA), which represents all DNA in the bloodstream and is not necessarily of tumor origin. This finding most likely reflects the tumor heterogeneity in these patients, which can be an important confounding factor for therapy. Remarkably, patients with more than one *AR* mutation had a poor response to Enzalutamide (Wyatt et al., 2016). This suggests that implementing *AR* mutation detection in patients prior and during the treatment would yield valuable prognostic information, and possibly influence clinical decision-making.

Below we will discuss in more detail the functional consequences of specific *AR* mutations detected in liquid biopsies (summarized in Table 1.).

4.2.1. Functional aspect of treatment-induced *AR* mutations

4.2.1.1. L702H

The L702H mutation was first identified in mCRPC patients receiving anti-androgens supplemented with glucocorticoids (Suzuki et al., 1993). Mechanistically, the hydroxyl group at position 17 α of the steroid molecule is critical for activation of AR L702H (van de Wijngaart et al., 2010). Notably, L702H may co-occur with T878A in the same allele. This double mutant AR was seen to be even more responsive to glucocorticoids (Matias et al., 2002).

More recently, Carreira and colleagues (2014) were able to detect L702H mutation in plasma DNA of up to 20% progressing after receiving glucocorticoids alone or in combination with abiraterone or other drugs. Notably, these patients were resistant to abiraterone that could be a result of activation of L702H mutation by glucocorticoids. and reduces the antagonistic effect of anti-androgenic compounds, although in transactivation assays both Enzalutamide and ARN-509 still inhibit the L702H mutant AR, however competitor used in these experiments was R1881 not prednisone (Lallous et al., 2016).

4.2.1.2. W742C

The AR W742C mutation was first detected in Bicalutamide-resistant mCRPC (Hara et al., 2003). Bicalutamide binding to wild type AR induces an improper movement of H12, thus preventing the recruitment of co-regulators. However, the change of

tryptophan to cysteine, allows Bicalutamide to adopt a different conformation within the LBD, allowing hydrogen bonding between the drug and the residues in the ligand binding pocket that resembles the one seen for DHT (Bohl, Gao, Miller, Bell, & Dalton, 2005). As a consequence, H12 is repositioned and the correct AF2 surface is exposed for co-activator recruitment. Importantly, both Enzalutamide and Abiraterone can effectively inhibit this mutant AR (Richards et al., 2012).

4.2.1.3. H875Y and T878A

The H875Y and T878A are the most frequently reported AR mutations. They too render the AR promiscuous for other steroid hormones. In case of T878A, the resulting enlargement of the ligand binding pocket allows binding and activation of the receptor by various steroids as well as the antagonist Flutamide (C.-Y. Chang, Walther, & McDonnell, 2001; Steketee et al., 2002; Zhao et al., 2000). For H875Y, a more indirect mechanism of action has been proposed via changes of the conformation of the ligand binding pocket. From the clinical perspective it is important to note that prednisone is effectively able to activate both these AR mutants (Richards et al., 2012).

In light of the more novel AR targeting therapies, it was observed that H875Y and T878A mutations are enriched in cfDNA of patients who were put on Abiraterone, but were also seen to be persistent in Enzalutamide treated population (Azad et al., 2015; Wyatt et al., 2016). The explanation might come from the fact that patients receiving Abiraterone also receive Prednisone and even when these patients switch to Enzalutamide they will frequently continue co-therapy with prednisone. Prednisone plus the other circulating androgens might thus be able to keep the AR signaling active and aid survival of cancer cells that harbor these mutations (Wyatt et al.,

2016). Therefore, it seems that mutations of certain residues that lead to promiscuity of the AR might be the driving force of resistance towards anti-androgens. The clinical implication, suggesting that better synthetic oral glucocorticoids that do not bind the mutant AR should be developed.

4.2.1.4. F877L and F877L/T878A

The F877L mutation was described first in an *in vitro* screen for AR mutations that convert Enzalutamide to an agonist (Balbas et al., 2013). Subsequently this mutation was detected by means of the BEAMing PCR technique in the circulation of patients who progressed on ARN-509 treatment (Joseph et al., 2013). However, there is little evidence for a withdrawal syndrome for Enzalutamide and the single F877L mutation is yet to be detected in this patient population (von Klot, Kuczyk, & Merseburger, 2014). Recent papers show that the F877L mutation fully converts Enzalutamide to a strong partial agonist only if it co-occurs with the T878A mutation in the same allele (Bogner, Zolghadr, Hickson, Romer, & Yurlova, 2016; Lallous et al., 2016; Prekovic et al., 2016). Strikingly, not the single F877L mutation but the double mutant has been found after Enzalutamide treatment (Azad et al., 2015). Other anti-androgens such as Bicalutamide, Abiraterone acetate and Galeterone are still effective against both the F877L and F877L/T877A mutant ARs (Prekovic et al., 2016).

4.2.1.5. S889G, M896V and T878A/S889G

Novel mutations in the vicinity or within H12 were found in CTCs of patients who progressed on Bicalutamide (Lallous et al., 2016) Both M896V and S889G were shown to lead to promiscuous behavior of the receptor, since Flutamide and Bicalutamide can activate these receptors. As discussed before, H12 is an important

player in the AR transactivation, since upon ligand binding the helix forms a scaffold for co-activator binding (Hodgson, Shen, Hollenberg, & Balk, 2008). The M895V mutation is proposed to lead to an enlarged ligand binding pocket, thus allowing Bicalutamide again to fit inside the pocket and activate the receptor. On the other hand, the S889G seems to have a more indirect mechanism. The introduction of a flexible glycine side chain at this position could influence the mobility of H12 and thus facilitate the formation of AF2 (Lallous et al., 2016). Importantly, while these single S889G and M895V mutations are still inhibited by Enzalutamide, the T878A/S889G double mutant appeared in the cfDNA after Enzalutamide progression. In vitro transactivation assays showed that this double mutant indeed converts Enzalutamide to a partial agonist (Lallous et al., 2016). With the F877L/T878A being previously described in Enzalutamide resistance, this poses a question whether double AR mutations will be found as common mechanisms in multiple-therapy resistant CRPC?

4.3. AR splice variants in the circulation

The splice variant AR-V7 has been detected in CTC after ADT, Abiraterone or Enzalutamide treatment and it was shown that the presence of AR-V7 associates with worse outcome on AR targeting agents for CRPC (Antonarakis et al., 2014; Steinestel et al., 2015). Moreover, higher levels of AR-V7 were seen in CTCs of patients with multiple prior AR targeting therapies, which seems to confirm that the tumors acquire this splice variant during therapy, however this should be confirmed on a larger cohort of patients (Steinestel, Luedeke, Arndt et al., 2015). Importantly, the presence of AR-V7 in CTCs was associated with inferior prostate-specific antigen (PSA) response and poor progression-free and overall survival (Nakazawa, Lu, Chen et al., 2015). A recent study suggests that CTC based AR-V7 mRNA detection could

predict outcome in patients with CRPC receiving first- and second-line hormonal therapy by using three separate prognostic categories (CTC-, CTC+/AR-V7-, and CTC+/AR-V7+) (Antonarakis et al., 2017).

Nakazawa *et al.* demonstrated that there is a high temporal variability in AR-V7 levels in CTCs of Abiraterone or Enzalutamide treated patients (Nakazawa et al., 2015). On the other hand, releasing the pressure from AR targeting by changing the treatment to taxanes resulted in a dynamic decrease in AR-V7.

Besides AR-V7, other splice variants also might play a role in therapy resistance in CRPC. Indeed, RNA sequencing of CTCs from patients with mCRPC revealed a complex and heterogeneous pattern of AR splice variant expression. The most abundant splice variants were AR-V7 and AR-V12. Furthermore, the majority of the patients had CTCs with simultaneous expression of more than one type of AR splice variant (Miyamoto, Zheng, Wittner et al., 2015).

While splice variants cannot be detected in ctDNA, it is possible to detect the transcripts in circulating RNA (El-Hefnawy et al., 2004). In contrast to CTC studies, standard PCR based techniques on circulating tumor RNA can more readily be transformed in a routine application.

4.4. AR axis activity in CTCs

Another promising approach is to use the subcellular localization or AR activity in CTCs as a predictive marker for clinical response. Docetaxel, a commonly used taxane, facilitates microtubule assembly, preventing depolymerisation which in-turn causes mitotic arrest and apoptosis (Yvon, Wadsworth, & Jordan, 1999). Besides this, as the nuclear transport of AR is dependent on microtubules, docetaxel partially

acts via inhibiting the AR signaling (Zhu et al., 2010). Indeed, in CRPC patients that underwent taxane therapy, a sustained cytoplasmic sequestration of the AR was seen in CTC from responders, while in CTC from non-responders the AR was also found in the nucleus (Darshan et al., 2011).

The CTCs were shown to be a useful tool for getting a direct readout of the AR signaling axis (Miyamoto et al., 2012). Even though this kind of experimental approach needs to be validated, the results hint that it provides insight into the response of the patients to inhibitors of the AR axis. Interestingly, CTCs originating from castration-sensitive PCa have predominantly an active AR signature, while the CRPC derived CTCs show large heterogeneity, with only a small portion of cells with an active AR signature, and abundance of either mixed or no AR signature. These results suggested that AR signaling is altered in CRPC, and that other signaling pathways such as Wnt or glucocorticoid receptor may contribute to diseases progression and therapy resistance (Arora et al., 2013; Miyamoto et al., 2015; Robinson et al., 2015). Importantly, some of the CRPC patients who had CTCs with active AR signature showed more than 50% decline in this CTC subset within 2-5 weeks on Abiraterone acetate (Miyamoto et al., 2012). Patients that had ligand-independent AR gene activation showed an increase in the number of CTCs with active AR signature during Abiraterone acetate therapy and a decrease in overall survival, pointing to variability in response even if the AR axis is still active.

4.5. Biosynthesis of androgens

The suppression of gonadal androgen synthesis by LHRH agonists or antagonists does not completely suppress androgen synthesis and AR signaling (Hu, Denmeade, & Luo, 2010; Laurent et al., 2016). As explained above, the low levels of androgens

are sufficient to activate AR when it is overexpressed. Moreover, CRPC cells can bypass the low androgen levels by producing DHT themselves (Cai et al., 2011). Importantly, overexpression of both AR and steroidogenic enzymes has been observed following Abiraterone therapy (Cai et al., 2011; Mitsiades et al., 2012). Thus, the question is whether we could detect these changes early enough to use them as biomarkers for the sensitivity to androgen biosynthesis inhibitors, or even for the application of a more focused therapeutic approach by targeting the biosynthetic enzymes involved? Using CTCs, Mitsiades and colleagues have shown that the expression of steroidogenic enzymes correlates with the tumor response to Abiraterone therapy (Mitsiades et al., 2012). Increased expression of enzymes involved in production of DHT and decreased expression of enzymes that degrade/inactivate DHT, alongside AR overexpression, was observed in CTCs of patients with CRPC. This could suffice to keep the AR signaling active despite therapy. In addition to this, Mitsiades and colleagues (2012) also describe a switch in expression from type-II 5 α -reductase (SRD5A2) to type-I (SRD5A1) in PCa. This knowledge could have a major impact on the selection of therapy as selective inhibitors were developed for these enzymes (Xu, Dalrymple, Becker, Denmeade, & Isaacs, 2006).

From a clinical perspective, profiling of therapeutic targets like the AR and the players involved in androgen signaling clearly could serve as a valuable tool in predicting drug efficacy, in studying how the resistance develops and in the optimization of sequencing of different therapeutics.

5. Conclusions

Liquid biopsies are a promising source of non-invasive information on tumor cell biology. The molecular characterization of the AR signaling axis in cfDNA or CTCs can be applied to monitor treatment responses in patients treated for mCRPC. However, further clinical testing is needed to assess in how far they provide correct and clinically useful information on the molecular mechanisms that underlie cancer aggressiveness and spread, therapy response, and development of resistance.

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Figure caption

Figure 1. Proliferation of prostate epithelial cells is dependent on the activity of the AR. While androgens (such as DHT) activate the receptor, antagonists such as Enzalutamide block its activity and thus inhibit growth of prostate cells. Blockade of the AR is used for prostate cancer treatment, and this therapeutic approach is at first effective. However, tumors of the patients receiving this line of therapy will eventually become resistant. The reactivation of the AR axis is a common mechanism of resistance and can occur via one of the ways depicted. AR gene can mutate generating a promiscuous receptor or leading to conversion of antagonists to agonists. Furthermore, the splice variants of AR lacking the LBD are seen to emerge after treatment with antagonists. Amplifications of the AR gene are also a commonly observed mechanism of AR axis reactivation in drug resistant prostate cancer. Another way of AR axis reactivation is through change in expression of enzymes involved in steroid metabolism leading to an increase in production of androgens within the tumor, which in the end leads to increase in AR activity despite low levels

of circulating androgens. These mechanisms behind anti-androgen resistance can be followed up in circulating cell free DNA or circulating tumor cells.

Table and table caption

Table 1. Overview of the AR mutations discussed in the review.

MUTATION	FUNCTIONAL CONSEQUENCE
L702H	AR responsive to glucocorticoids
W742C	Bicalutamide becomes an agonist
H875Y	AR activated by other steroid hormones
F877L	Enzalutamide becomes a weak partial agonist
T878A	AR activated by other steroid hormones; Flutamide becomes an agonist
F877L/T878A	Enzalutamide becomes a strong partial agonist
S889G	Flutamide and Bicalutamide become agonists
T878A/S889G	Enzalutamide becomes a partial agonist
M896V	Flutamide and Bicalutamide become agonists

Highlights

- Prostate cancer progression and treatment relapse is associated with changes in the androgen receptor axis
- Circulation is easily accessible source of both circulating tumor cells and circulating tumor DNA
- Androgen receptor axis alternations detectable in the blood could be of potential use in therapeutic decision-making and precision medicine for metastatic castration-resistant prostate cancer