Molecular Mechanisms Involved in the Transition of Prostate Cancer Cells from Androgen Dependant to Castration Resistant State

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Keywords: Prostate Cancer; Androgen Dependant; Castration Resistant; Transition

Abstract

Prostate cancer is an internationally important health problem of the man, particularly in developed countries. Currently the main line of treatment for prostatic cancer is based on androgen deprivation. The androgen blockade treatment is initially effective on androgen dependent cancer, but the disease relapses within a period of around two years with a more aggressive form named castration resistant cancer. The growth and expansion of castration resistant cancer are not dependent on androgen supply anymore and thus is more difficult to treat. Understanding the molecular mechanism involved in the transition of prostate cancer cells from androgen dependent to androgen independent state is the key for designing a biologically appropriate strategy for a more effective treatment. Currently, several different theories have been brought about to address this transition process and based on these theories, several lines of treatments were introduced for the castration resistant disease. Despite the advances in our knowledge of prostate cancer cell transition, no current theoretical explanation can satisfactorily cover all aspects of this transition process and no curative treatment of the castration resistant disease is available. In this review, we summarized and commented several major current theories to explain the molecular pathology on the transition of prostate cancer cells from androgen dependent to androgen independent state. In addition, we have also briefly introduced an alternative mechanism to explain this complicated transition process.

Introduction

Prostate cancer is the second most common male cancer worldwide, and the fourth most common cancer overall, with more than 1,111,000 new cases diagnosed in 2012 (15% of male cases and 8% of the total).Prostate cancer (PCa) is the most common cancer in men in Europe. In the UK, it accounts for approximately a quarter (24%) of all new male cancer diagnoses with 41,736 men are diagnosed with prostate cancer each year (Cancer Research 2011). In the UK, an average of 36% of cases were diagnosed in men aged 75 years and over, and only 1% were diagnosed in the under-50s. The incidence rate was 104.7/100,000 and mortality rate was 23.7/100,000 in 2011 (http://www.cancerresearchuk.org).

Prostate cancer is a hormone driven cancer which has been discovered in the 1940s by Huggins and Hodges [1]. Androgen is the key player in regulating the development and maintenance of male characteristics through binding to its receptors. The predominant and most active androgen is testosterone which is mainly (approximately 90%) synthesized by cells in the testes and

an important factor in the development and function of the prostate gland. Androgen receptor (AR) is a steroid receptor and a member of the larger nuclear receptor family which act as a transcription factor after binding to various binding sites. It is located on Chr Xq12 and made of 8 exons and has 8 splice variants (Figure 1) and it consists of a DNA binding domain (DBD), a ligand-binding domain (LBD), two transcriptional activation domains and a hinge region which introduce nuclear localization [2-4]. In the absence of androgen, AR is located in the cytoplasm with chaperone proteins such as HSP90, HSP40 and HSP23 [5]. When androgen binds to the ligand binding site, a conformational change will happen in the structure of the gene. The AR then transfer to the nucleus and forms a homodimer which then bind to the regulatory regions of other genes that are critical for the normal function and differentiation of prostate [5]. The genes regulating AR may be critical in the development of PCa but their role is not completely clear.

Androgen- deprivation therapy (ADT) is the main treatment for localized PCa. Although the initial response to treatment is very good but the relapse of a more resistance and aggressive type of cancer is inevitable and invariable. Although the castration resistance cells are androgen independent, it is clear that signalling through AR continues to be effective for tumour growth under castrations condition. This is called castration-resistant prostate cancer (CRPC) which is still signalling through AR but with different mechanisms. CRPC is the second most common cause of death in American men and currently is irredeemable [6].

As the CRPC is still depending on AR signalling, the current treatments are not targeting the receptors directly, but work indirectly to either reduce androgen or block the ligand binding site in the AR gene. One group of these drugs is CYP17 inhibitors (abiraterone, ketoconazole, orteronel, VN/124-1) which inhibit the enzyme that bio-synthesis androgen from its precursor [7]. MDV3100 (enzalutamide) is another group of these drugs and is a
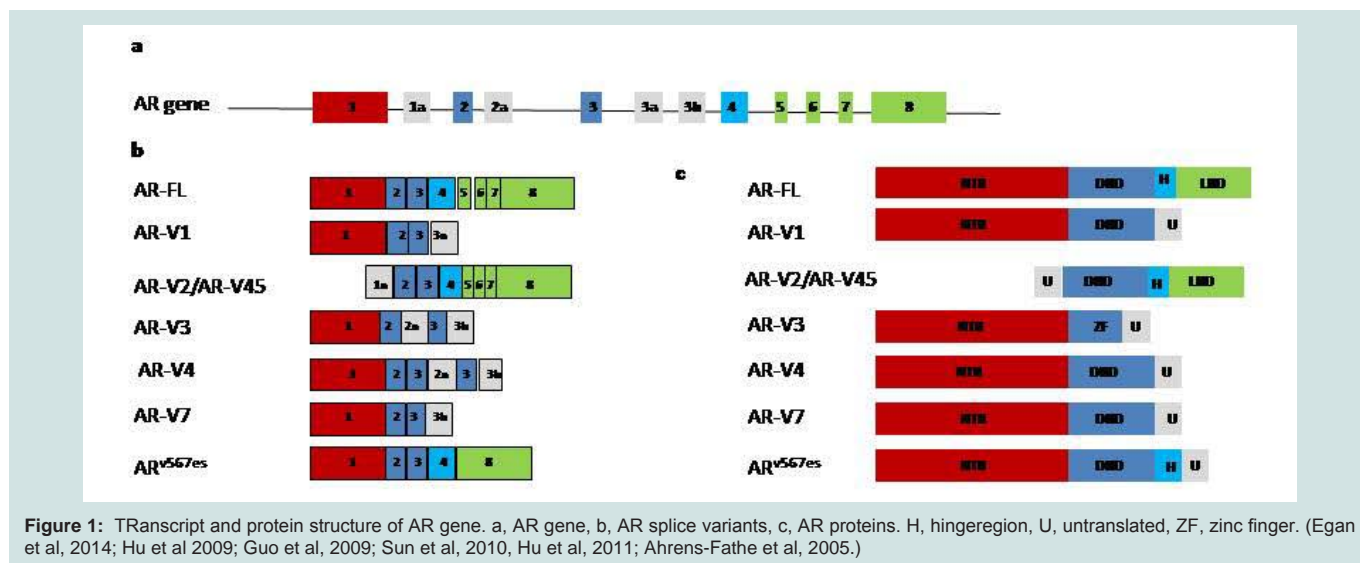
Figure 1: TRanscript and protein structure of AR gene. a, AR gene, b, AR splice variants, c, AR proteins. H, hingeregion, U, untranslated, ZF, zinc finger. (Egan et al, 2014; Hu et al 2009; Guo et al, 2009; Sun et al, 2010, Hu et al, 2011; Ahrens-Fathe et al, 2005.)

novel anti-androgen drug which compete with androgen for binding to ligand-binding site in AR [8]. In this review, we summarized and commented several major current theories to explain the molecular pathology on the transition of prostate cancer cells from androgen dependent to androgen independent state. In addition, we have also briefly introduced an alternative mechanism to explain this complicated transition process.

Why PCa cells become castration resistant

Amplification and/or overexpression of AR

AR is expressed in both mRNA and protein level in all PCa patients but its overexpression in CRPC may be due to long term ablation of androgen. There is an increase in expression of AR in both mRNA and protein level, which is due to the amplification of this gene [9]. Chen 2004 demonstrated the overexpression of AR in PCa xenograft and its transformation from androgen dependant to androgen independent. Also they reported that AR overexpression can change the AR antagonist to agonist [10]. Amplification of AR gene has been reported in 30% of CRPC cases and gain of copy number has been reported in 80% of CRPCs [9,11,12]. The amplification was rarely detected in un-treated PCa [9]. High levels of AR amplification have also been detected in 38-63% of circulating tumour cells in CRPC patients with metastasis [13,14]. Their respond to the second line of androgen blockade is also good [15]. Other causes of overexpression of AR may be deregulation of miRNAs which regulate AR or shortened 3'UTR in AR gene [16]. For example down-regulation of miR-let-7c is inversely correlated with AR expression but Lin28 is positively correlated with AR [17]. Also the deregulation of transcription factors such as NF-κB has been detected in CRPC and is correlated to AR overexpression [18]. NF-κB binds to the promoter region of AR and increased both mRNA and protein and NF-κB inhibitor can reduce the AR and tumour growth. Another factor that has been correlated to overexpression of AR is loss of retinoblastoma protein (RB1). This will increase the expression of transcription factor E2F1 and cause increase transcription of AR [19].

Mutation of the AR gene

Prostate cancer is a highly heterogeneous disease which will be achieved by a subset of cell that acquired additional mutations after intrinsic and extrinsic stimuli which promote their aggressiveness and metastasis [20,21]. AR has the most mutations among the hormone receptors with >660 mutations reported. Mutations in AR gene are rare in untreated PC but more in CRPC which develop in 10-30% of patients after the treatment. Mutations are mainly occurring in NTD (37%), LBD (40%) and DBD (9%) region of the AR gene and they may result in the activation of AR with weak adrenal androgens or they may convert AR antagonist to AR agonist in which can activate tumour growth. One of the most common mutations in AR which affects the ligand specificity of AR is T877A. In this mutation an alanine is replaced by a threonine [22]. This mutation alters the stereochemistry of the binding pocket and allows other nuclear hormones, corticosteroids and anti-androgens to activate the AR[23,24]. Another one of these mutations called F876L which will reverse the anti-androgenic effect of enzalutamide to an agonist of AR[25]. The treatment plan for these patients is to target the downstream effectors of AR signalling and/or use other anti-androgen drugs such as bicalutamide [25]. Several other mutations in LBD have been detected such as L701H, H874Y, V715M, V730M and W742C which increased the sensitivity of AR to other steroids. L701H mutated cells are highly sensitive to glucocorticoids at physiological concentrations [26,27]. H874Y was originally detected in CWR22 xenograft and CRPC patients treated with flutamide. They are more sensitive to hormone and other steroids. This mutation is far from LBD and its effect is due to conformational changes in AR protein [24,28]. V715M, V730M and W742C are rare mutations in CRPC and increased the sensitivity of AR to low concentrations of androgen and also adrenal androgen. The tumour growth has been increased in tumours with W742C mutation which received bicalutamide and flutamide [29,30]. Therefore, this mutation may convert antagonist to agonist.

Co-Regulators of AR

The regulatory role of AR in the nucleus has been introduced through a series of co-regulatory proteins [31]. These co-regulators

can either enhance (p160/SRC and CBP/p300) or suppress (NCoR and SMRT) the transcription through alteration in ligand specificity or activation of AR with low levels of androgen [32]. The deregulation of co-regulators can result in the development of CRPC [33]. The co-regulators have different functions, some are chaperons (HSP90), some are enzymes which modify histone, SW1/SNF alters chromatin and HATs modify histones post-transcriptionally [31,34]. It has been reported that increased expression of SRC [encoding v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog] family plays a role in both AR-dependent and AR-independent PCa. SRC-1, SRC-2 and SRC-3 have been bound to NTD in AR and activated it via histone acetyltransferase activity. Amplification of SRC-2 has been detected in 11% of PCa which may increase the sensitivity of AR to weak agonists [12]. The level of SRC-2 is reduced by androgen; therefore using ADT will increase the SRC-2 [35]. Phosphorylated SRC-1 can activate AR in the absence of androgen with the same scale. Elevated level of SRC-3 is associated with a more aggressive form of the disease [36].

Forkhead box A1 (FoxA1) is a member of the forkhead family of transcription factors and they interact with chromatin as a pioneer factor in nuclear receptors [37]. Therefore, absence or deletion of FoxA1 can cause loss of AR binding to chromatin. From other hand it has been shown that knocking down FoxA1 in prostate cells resulted in redistribution of AR binding site and gain in novel binding domains [35]. This suggested the double sword effect of FoxA1 either as facilitator or trans-repressor of AR binding to chromatin [35,36]. This gene is important in the development of CRPC.

Another protein acts as co-activator is TFF3 (trefoil factor 3), which is differentially expressed in native PCa compare to CRPC and it is involved in the ERG (v-ets erythroblastosis virus E26 oncogene homolog) rearrangement. TFF3 is directly regulated by ERG in both types of PCa. ERG inhibits the expression of TFF3 in the presence of androgen but stimulate it in the androgen free environment [37]. This shows the double effect of ERG on the regulation of TFF3 which depends on the level of androgen in the microenvironment of cancer. Induced TFF expression by ERG enhanced the invasiveness of CRPC cells which may be due to switching on an AR suppressed pathway [37]. ERG can also function independently of AR signalling in prostate cancer. Overexpression of ERG in PCa is associated with increased invasion and proliferation [38]. It has been shown that increased ERG in the presence of aberrant PI3K pathway will induce invasiveness of PCa [39].

Enhancer of zeste homologue 2 (EZH2), a catalytic subunit of polycomb repressor complex 2 (PRC2), has been involved in the progression of PCa [40]. In prostate cancer the expression of EZH2 is higher in more aggressive cancer and high expression is correlated to poor prognosis [41]. EZH2 is known as a transcription repressor, but it has also acted as a transcription activator. The transformation from a repressor to an activator is modulated by phosphorylation of serine-21 which is regulated by PI3K/Akt pathway [42]. Phosphorylation makes the cells independent of androgen and this can be responsible for the development of CRPC.

Cyclin D1b is another co-regulator of AR. Cooperation of AR and Cyclin D1b results in the induction of Slug (SNA12), a member of the SNAIL family of transcriptional factors [43]. Slug is a known

gene in the induction of epithelia-mesenchymal transition in PCa and it was reported that knocking down slug suppressed the invasive capacity of PCa especially in cells overexpressing Cyclin D1b [43]. Also Slug has been reported as an AR co-activator which can enhance AR transcriptional activity in CRPC. This works through a positive feedback mechanism via slug in which cooperative signalling between Cyclin D1b and AR can increase AR functional activity [38].

AR Splice Variants

Alternative splicing is a common feature in the cancer and there are numerous splice variants that are associated with progression and metastasis of cancer. The splice variants somehow adjust themselves to be free of growth factors and suppressor genes and they can cope easier with hypoxia. This will enable them to escape immune system and speared in the body. In PCa the late native splicing has been detected in a number of genes. AR is one of them and alternative splicing of AR has not been detected in normal prostate tissue [39-41]. The most common feature of alternative splicing is the N-terminal Domain (NTD) and DNA binding Domain (DBD) and disrupted ligand binding domain (LBD) which will cause resistance to drugs (Figure 1). The mechanism of splice variant is not clear. There are reports of alternative initiation of translation which results in shorter AR [42]. The other hypothesis is the proteolytic cleavage of full length AR which is a post-transcriptional mechanism [43]. RNAi studies show that by targeting the LBD, only the full length is suppressed and the shorter variants are still active [44]. AR amplification and deletion can also result to alternative splicing. Deletion in exon 5, 6 and 7 can generate a truncated isoform. The most common variants detected in CRPC are AR-V7 and AR^{v567es} and high expression of these variants are correlated with poorer patient's survival [45]. AR-V7 has been found in tissue samples of CRPC patients and CRPC cell line (22RV1) which lack LBD but contain 16 unique amino acids [39,40]. In pre-clinical models, levels of AR-V7 cannot be suppressed and may be increased by abiraterone and enzalutamide [46,47]. Gene expression profiling of variants knockdown cells show that AR-Vs and AR-FL regulate distinct characteristic set of target genes [47,48]. Up-regulation of AR-Vs in CRPC may be due to an adaptive mechanism to the androgen axis treatment. Although the presence of AR alternative splices is a venue of interest in prostate cancer, lack of antibody for specific isoforms restricts the studies. The RNA level does not always reflect the protein level. It has been reported that AR-Vs in mRNA level may be low in CRPC patients, but their protein level could express 32% of the AR-FL [45]. Following the resistance to abiraterone and enzalutamide in the CRPC, the presence of a number of truncated AR splice variants has been reported in CRPC patients [40,49]. The need for N-terminal inhibitors is urgent to determine if AR-Vs are contributing mediators of CRPC or biomarkers of aggressiveness. However, until N-terminal inhibitors of the AR are available for clinical use, it is unlikely the question will be resolved experimentally [49].

Changes in Cell Signalling Pathways that Modulate AR Function

Changes in steroid metabolism within the tumour cells: Testosterone produced in the testes can be converted into its active form, dihydrotestosterone (DHT), in the prostate. Although ADT reduce the amount of testosterone in CRPC patients, the DHT in

the tissue is high enough to activate the AR. This may lead to an endogenous synthesis of DHT [50]. It was reported that in CRPC the increased level of transcripts encoding enzymes involved in androgen metabolism can cause intra-tumoral production of DHT [51].

Bypass AR pathway by other steroid receptors: The role of glucocorticoid receptors (GR) has been studied in CRPC and it has been reported that glucocorticoids can slow down disease progression in CRPC. This can be achieved through suppression of adrenal gland activity and also through changes in TGF- β, IL-6 and IL-8 which have anti-proliferative effect. Increased expression of GR is due to a bypass system after blockade of androgen and this can cause resistance to anti-androgen drugs such as enzalutamide [37,52]. There is a high homology between the six steroid receptors (AR, ESR α, ESR β, PR, GR and mineralocorticoid receptor) especially in their DBD. Therefore, further elucidation is required to see the relationship of other steroid receptors to the CRPC. In CRPC, DHT can be synthesized from cholesterol. There are reports of up-regulation of the steriodogenic acute regulator protein (StAR) in CRPC patients. This protein regulates the transfer of cholesterol to mitochondria to activate the steroidogenesis pathways [53].

RSK/YB-1 signalling: AR has been overexpressed in most of the CRPC cases, with amplification responsible for only 10-20% of these cases. The alteration in AR signalling is another reason for AR overexpression. One of such involved signalling pathways is RSK/YB1 pathway. Y-box binding protein-1 (YB-1) is a poleiotropic factor that binds to the Y-box sequence (5'ATTGG 3') in nucleus and modulates gene specific translation. YB-1 is up-regulated in prostate cancer and it is also correlated with androgen ablation and tumour progression, thus plays a role in the progression of PCa to CRPC [54]. It has been shown that YB-1 binds to AR promoter and regulates AR transcription through the promoter region. YB-1 is highly expressed in CRPC both *in vitro* and *in vivo*. It has been shown that YB-1 is translocated to nucleus due to cellular stress by phosphorylation. Furthermore, AKT and p90 ribosomal S6 kinases (RSK) are responsible for YB-1 phosphorylation [55,56]. The RSK family of Ser/Thr kinases consist of four isoforms and RSK1 and RSK2 are overexpressed in human prostate cancer.It has been reported that blocking AR signalling either by androgen depletion or treatment with anti-androgen agent caused the activation of RSK/YB1 signalling pathway which can induce AR. Inhibition of this pathway can suppress AR induction and the growth of prostate cancer [57]. Using SL0101 (a RSK inhibitor) to inhibit tumour initiation by inactivating YB-1 and combination of this inhibitor with enzalutamide in CRPC will increase and prolong the response to treatment [57].

Post-Translational Modification of AR

mircoRNA: miRNAs are short (21-23 bp) non-coding RNAs which act as transcriptional or post-transcriptional regulators of gene expression. Recent studies have reported the use of these RNAs for diagnosis and prognosis of cancer. The first miRNA reported in serum of patients with CRPC was mir-21. The serum expression level of mir-21 was significantly higher in CRPC compared to androgen dependant and localised prostate cancer [58]. Other miRNAs with high expression levels in serum and tissue of CRPC patients are miR-141, miR-298, and miR-375 [59]. mir-141, miR-298 and miR-375 are

significantly elevated in serum of metastatic CRPC than localized PCa and Mir-141 and miR-375 are also elevated in the tumour tissues [59]. MiR-221/-222 is increased in CRPC and miR-23b/27b is repressed in CRPC [60]. Suppression effect of miR-23b/27b is obtained through reducing Rac1 activity and increasing E-cadherin level [61]. Another AR and CRPC related miRNAs are miR-124 and miR-125b. miR-124 has been reduced in prostate cancer compared to BPH which is due to hypermethylation. It acts as a direct target of AR by down-regulating miR-125b and up-regulation of p53 [62]. Therefore miR-124-AR-miR-125b pathway was introduced as a potential novel target. Using anti-mir-125b has resulted in apoptosis induction in both androgen dependant and independent PCa by affecting on p52, Puma, bak1 and p14^{ARF}, releasing mitochondrial CytC, SMAC and activation of Cas3 [62,63]. To examine the tumour growth *in vivo,* mice was injected with a transfected cells with high levels of mir-125 (19-fold greater). Tumour growth was much faster than control and after castration, there was a slight regression. When 22RV1 cells were transfected with lento-miR-124 (23-fold increase), tumour growth was inhibited and AR was lower compared to the control [62].

Anti-miR-125b can sensitize PCa to cisplatin or genistein treatment. Inhibition of miR-125b will increase p53 which is essential for docetaxel sensitivity of PCa [64]. Mir-30 is also important in CRPC and is involved in the Src tyrosine kinase activity. Induction of miR-30 will inhibit the growth, invasion and migration of CRPC cells [65]. miR-30 binds to ERG at 3' UTR and can affect the downstream targets of EGR such as c-MYC [66].

lncRNA: Human genome produces both protein coding and non-coding RNAs but the effect of non-coding RNA was underestimated. During the last few years more attention has been made to identify lncRNA, as 90% of the human genome transcripts are consisted of non-coding RNAs. Therefore revealing the role of lncRNAs in cancer can be of great promise for the early detection, prevention and treatment of tumours. lncRNAs are RNA molecules >200bp long. They are frequently polyadenylated and associated with transcription by polymerase II [67].

MALAT-1 is a lncRNA and it is involved in regulation of metastasis and motility in cancers. It is located on Chr 11q13 and consists of more than 8000 nt. The expression of MALAT-1 in prostate cancer tissue was much higher than in the normal counterpart and among cancer samples, CRPC tumours were demonstrated much higher expression.Suppression of MALAT-1 results in reduction of the growth of CRPC tumours and metastasis [68].

Another lncRNA which has been confirmed to be involved in the transition of prostate cancer from androgen-dependent to androgen independent form is linc00963 which is located on Chr 9q34.11. Using the knockdown strategy, the function of linc00963 on cell proliferation, apoptosis, migration and invasion were evaluated in highly malignant C4-2 cell line. It was confirmed that the transition was via the EGFR signalling pathway [69].

The 3rd lncRNA located in the PCAT-114 gene is SChLAP1 (Second Chromosome Locus Associated with Prostate-1, also called *LINC00913*). The size is 1.4kb and it composes of up to seven exons. It has been reported that SChLAP-1 was highly expressed in around 25% of prostate cancer and it is more frequent in high Gleason score and associated with EST fusion [70]. This has been associated with

the progression of prostate cancer and metastasis. High expression of SChLAP1 is associated with poor prognosis of patients. It has been confirmed that SW1/SNF has an opposite correlation with SChLAP1, as loss of SW1/SNF promotes cancer progression. Therefore, high SChLAP1 has an antagonistic effect on SW1/SNF [70].

Prostate Cancer Associated Transcript-1 (PCAT-1) is another lncRNA which is located on Chr 8q24, and approximately 725kb upstream of c-MYC. PCAT-1 is highly overexpressed in high grade and metastatic prostate cancer. There is a direct correlation between the PCAT-1 and EZH2 expression in high grade patients [71]. It has been reported that PCAT-1 is involved in the double strand DNA break (DSB) [72].

Role of Stem Cells in CRPC

Recently, it has been indicated that cancer stem cells can play a role in the epithelial-to-mesenchymal transition (EMT) which results in drug resistance [73]. Through EMT, epithelial cells lose cell polarity, cell-cell adhesion, and gain mesenchymal characteristic such as high capability of migration, invasion, anti-apoptosis and disarrangement of extracellular matrix. In prostate cancer, castration will induce EMT [74] which may cause the cells to leave epithelium and invade distal organs. There are a number of proteins involved in the EMT process such as E-cadherin, N-cadherin, Vimentin, snail, Zeb1, Zeb 2, TWIST and Slug [75]. E-cadherin is located in the surface of epithelial cells and facilitates cell-cell adhesion in normal epithelium. In most cancer, E-cadherin is reduced which can drive to EMT. Snail, Slug, Zeb1, Zeb2 and TWIST reduce E-cadherin. N-cadherin and Vimentin are associated with the initiation of EMT and progression to invasive form [76]. In CRPC the expression of Zeb1 and TWIST is much higher than the ADT PCa and blockade of Twist will increase E-cadherin level [77]. Slug, an EMT transcription factor is overexpressed in CRPC and promotes tumour development [78]. High expression of N-cadherin has been reported in primary and metastatic tumors of patients with CRPC and specific antibodies against N-cadherin can suppress tumour growth, metastasis and invasion through reduction the activities of Akt and IL-8 [79].

Cancer stem cells (CSCs) are stem-like cells in tumours and have ability to grow and differentiate to different tumour cells. They have specific surface antigens and retain mesenchymal phenotypes which is important in progression to CRPC [73]. It has been proposed that androgen independent cells are located in basal layer and the number of these androgen independent cells is very small compared to androgen dependant cells. After androgen ablation the androgen sensitive cells are destroyed but the androgen independent cells survived and become dominant CRPC [80,81]. There are a number of biomarkers correlated to CSCs in CRPC such as CD166, Sox2, Lgr4, Sca-1, CD44, p63 and etc [73]. There are also a number of pathways involved in the EMT and CSCs toward CRPC such as AR pathways [82-84], growth-factor receptor tyrosine kinase activated pathways [85-87], Pten related pathways [88], STAT3 related pathways [89,90], Wnt pathway [91-93], Notch and Hedgehog pathways [94-96].

Role of fatty acids in CRPC

Fatty acids (FA) and cholesterol have many regulatory functions in living cells and they are main precursors for lipids and have proven roles in the development and progression of prostate cancer [97,98].

In Western countries the increased incidence of prostate cancer is associated with high consumption of omega-6 which is in red meat, refined vegetable oil and highly processed food [99,100]. On the other hand the Western diet lacks omega-3 which has been proven to have an inverse effect on the progression of prostate cancer [101,102]. It has been reported that maintaining a low omega6/omega3 ratio can prolong the respond of PCa to androgen treatment and delay progression to CRPC [103]. This effect is through affecting a number of pathways which are important in the proliferation, cell cycle progression and survival of prostate cancer cells. One of these pathways is PI3K/Akt/mTOR axis. High consumption of omega-6 will activate this pathway and the reduced ratio of omega-6/omega-3 will delay the progression of PCa in a dose dependent manner. NFκB is another transcription factor in the downstream of the Akt pathway which is increased significantly in CRPC. Reducing the ratio of omega-6/omega-3 will cause reduction in NFκB and prevent its translocation to nucleus, thus to reduce its transcriptional activity [104]. Cyclin D1 is another protooncogene involved in cell cycle and its overexpression has been associated with androgen independent cancer [105]. Reduction in the omega-6/omega-3 ratio will suppress cyclin D1 expression [103]. Another marker which has been changed by reduction of omega-6/omega-3 ratio is caspase-3 responsible for promoting apoptosis of cells. In CRPC cells this protein is reduced and therefore cells don't respond to treatment. By reducing the ratio of fatty acids the level of caspase-3 will increase and delay the progression of PCa to CRPC [103].

Fatty acids also stimulate steroid synthesis from cholesterol in steroidogenic organs [106,107]. SREBP, an AR regulatory transcriptional factor, is responsible for androgen synthesis and is increased in CRPC [108-110]. They are also responsible for the regulation of endogenous fatty acids and cholesterol and central precursors of androgen [51,108,111,112].

Another biomarkers involved is the fatty acid transport is C-FABP. The expression of this protein is high in androgen independent cells (PC3 and PC3M), low in androgen sensitive cell (22RV1) or none in androgen dependant cells (LNCaP) [113-115]. Fatty acids are sources of nutrition and energy. In weakly malignant androgen dependent PCa cells, relatively small amount of fatty acids is transported by C-FABP into cells and used as a source of nutrition. In highly malignant PCa and CRPC cells with a high level of C-FABP, large amount of intracellular fatty acidsis transported into the cells and the excessive amount of fatty acids can act as signalling molecules to stimulate their nuclear receptor, PPARγ. The activated PPARγ may trigger a series of molecular events that lead to a facilitated malignant progression through promoting angiogenesis and suppressing apoptosis [114]. As the increased cellular uptake of fatty acids transported by elevated levels of C-FABP in cancer cells was reported, the increased production of fatty acids was also evidenced by increased fatty acid synthase (FASN) in CRPC cells [116,117].

Conclusion

A number of mechanisms have been introduced for the transition of androgen dependent prostate cancer to androgen independent state (Figure 2). The most common mechanism is the amplification of the sensitivity of the AR due to androgen deprivation. Transcription factor and miRNA deregulation are also important mechanisms.
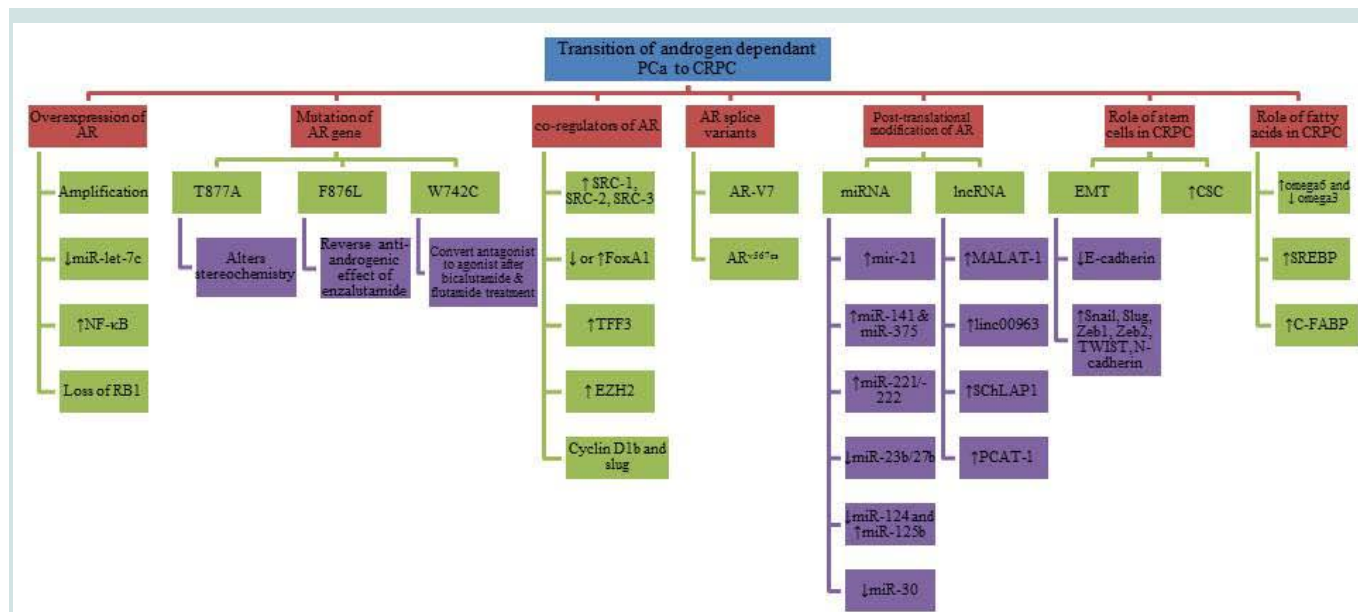
Figure 2: Summery of the mechanisms involved in the transition of prostate cancer cells from androgen dependant to castration resistant form.

When the cancer cells are deprived of androgen supply in the initial round of treatment, cells try to maximize their survival ability by increasing the AR sensitivity to make full use of a small amount of androgen remains. Therefore, some survived cells with an increased ability to use an epsilon quantity of androgen become dominant and castration resistant. Mutations are another adaptive mechanism which can change antagonist effect of drugs to agonist effect which can be harmful to patients and it has to be considered before the start of the treatment in CRPC cases. Another mechanism which has been highlighted was AR splicing variants which can cause resistance to abiraterone and enzalutamide in the CRPC. Post-translational modifications can affect the activity, stability, localization and interaction with other proteins. Although each current theory described above can explain certain aspects of the complicated molecular pathology involved in the transition of PCa cells from androgen dependent to androgen independent state, no single theory can satisfactorily explain every aspect. For example, if the AR sensitivity amplification theory is true, we would expect that re-expression of AR in AR-negative PCa cells could increase the malignancy. But several studies on the highly malignant PC3 cells showed that the forced re-expression of AR in PC3 cells actually reduced their malignancy [118-120]. Thus further study is needed to find out exactly what has made the PCa cell transition. The recent discovery of a novel tumorigenicity-promoting signalling pathway named C-FABP (fatty acids)-PPARγ-VEGF axis provided an alternative theory for the transition of PCa cells (Forootan FS, et al, in preparation for publication). This theory hypothesized that when the cancer cells are deprived of androgen supply in the initial round of chemotherapy, the cells are desperate to seek for new sources of energy supply and under the heavy selection pressure, most of the cells died from starvation. However, some cancer cells may have survived the pressure by switching their reliance on androgen to fatty acids (transported by C-FABP) as an alternative energy source. These cells are the so-called castration resistant cells. This hypothesis provided a new window of opportunity to observe this crucial issue in prostate cancer research from an entirely different angle.

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