



Prostate Cancer: Countdown to Choice between Stitch in Time and Eleventh Hour Begins

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Abstract: Androgen ablation therapy presumed to be an effective treatment for advanced prostate cancer (PCa) is relentlessly challenged. Remissions are impermanent and patients almost inescapably progress to become castration-resistant prostate cancer (CRPC). CRPC is almost invincible and is the major stumbling block in the treatment. It is a dramatic shift of androgen receptor (AR) from normal activities to the oncogenesis. AR signaling is remarkably increased under an androgen-depleted environment. It utilizes a miscellany of mechanisms and pathways to compensate for the decreasing levels of androgens. These range from mutations in the receptor more like a business tactic to attract more clients, to the illegitimate crosstalks which promote the signaling. The review will encompass various mechanistic insights of the AR manipulations. Moreover efficacy of therapeutic interventions recently designed keeping in view the molecular hierarchy will be evaluated.

Keywords: AR, miRNA.

Introduction

Prostate cancer (PC) cells remarkably express androgen receptor (AR) and need androgens to survive. Androgen suppression is doubtlessly, front-line therapy for metastatic disease. Almost all PC patients at the start show responsiveness to hormonal therapy but gradually show refractoriness to castration. There is an accumulating confirmation that these tumours are relying on AR signalling. Numerous mechanisms that augment AR signalling in an androgen-depleted environment have been explicated.

Prostatic carcinogenesis is a switch from the stromal-cell-dependent paracrine to an autocrine mechanism associated with AR-stimulated growth. As an important component of this conversion, AR undergoes switching from its capacity to suppress neoplasia of normal prostatic epithelia to directly stimulating the uncontrolled and un-differentiated population of prostate cancer cells. The following review will give a snapshot of the strategic manipulation of the androgen receptor to circumvent the androgen deprivation. These are well planned tactics at molecular level to maintain the dynamics of androgen receptor. It occurs at three levels primarily. At receptor level, at hormonal level i.e increasing the pleiotropy or integration at signaling level.

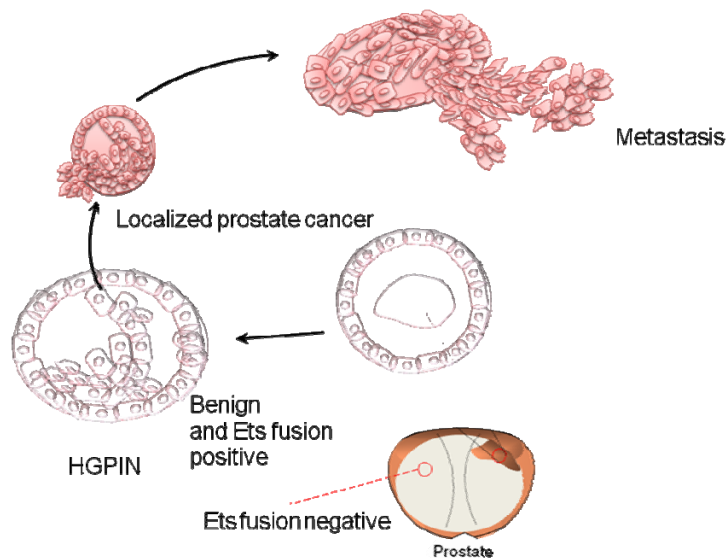


Fig. 1 An overview of the transition from localized to metastatic stage. Short term (Androgen Deprivation Therapy) ADT seems to be more effective than long term ADT with hazardous outcomes. An intermittent ADT is more reasonable in terms of clinical management.

Androgen receptor infrastucture: Marching to a different drummer

The biological multiplicity of prostate cancer is the major stumbling block in the standardization of therapy. Currently a paradigm shift has occurred in the clinical management of prostate cancer. Instead of long term androgen ablation/deprivation therapy (ADT), scientists have strengthened the concept of intermittent ADT (Fig. 1). Progress in molecular profiling suggests that divergence in the genetic profile of tumors considerably participate to the intricacy of the disease. Alternative pre-mRNA splicing is principal genetic process involved in biological diversity. During alternative splicing, coding and noncoding regions of a single gene undergo rearrangement to generate several messenger RNA transcripts yielding discrete protein isoforms with multifarious biological functions. Dysregulation of the splicing machinery influence splicing of cancer-relevant genes. It is interesting to note that androgen receptor undertakes aberrant and alternative splicing and gives rise to proteins that influence cell phenotypes and survival of patients. Splicing mechanisms must be manipulated at clinical level. In view of the fact that splicing is concerned with information transfer from the genome to the proteome, it incorporates another vital dimension to '-omics'-based molecular signatures utilized to individualize clinical management of patients.

Dehm et al [1], profiled the variants of androgen receptor in cell line. They were able to register full-length version of AR with duplicated exon 3 and two truncated versions lacking the COOH terminal domain (CTD). AR isoforms also exist in CTD-truncated version and are encoded by mRNAs that have a novel exon 2b at their 3' end. In a ligand-independent manner, AR isoforms promote the expression of endogenous AR-dependent genes, as well as the proliferation of 22Rv1 cells. However discordant finding was given by Marcias et al. [8], who documented a variant. 22Rv1 cells also express a mutant AR lacking exon 3 tandem duplication, doubtlessly, a major feature of this cell line. Guo et al. [5], registered ligand-binding domain deficient AR splice variants in hormone-insensitive PCA cells. AR3, one of the major splice variants expressed in human prostate tissues, is constitutively active and transcriptome was not triggered by androgens. At present available antiandrogen drugs are

unable to drug novel AR splice variants are not inhibited by. A rational drug design targeting these AR isoforms may potentially be successful for treatment of ablation-resistant PCA. Sun et al. [15], documented another novel human AR splice variant deficient in exons 5, 6, and 7 (ARv567es). This accumulating data marks these variants as candidates for therapies directly targeting the AR rather than ligand. CRPC cells express variant ARs which included truncated ARs (tARs), siRNA-mediated knockdown efficiently suppressed the androgen-independent cell growth. Nigericin-like compounds suppress AR expression at the mRNA level. This could be an approach for the clinical management of prostate cancer [9].

Androgen signaling: Many roads lead to Rome

It is a matter of deep concern for the cell to prolong the signaling of the cancer driving genes via AR. It ensures the robust expression and activation of androgen receptor mediated genes somehow or other. There are multidirectional pathways opted by the androgen receptor to meet the demands of desperate cancer prone environment. In the hypersensitive pathway, there is a robust quantitative expression of androgen receptor (AR) usually by gene amplification or AR has enhanced sensitivity to pay compensation for low levels of androgen or more testosterone is converted to the more potent androgen, dihydrotestosterone (DHT) by reductase. In the promiscuous pathway, criticalities are replaced by generalized patterns. The “specificity landscape” of the AR is broadened to encompass more non-specific ligands so that it can be activated by non-androgenic molecules normally present in the circulation. In the outlaw pathway, receptor tyrosine kinases (RTKs) are switched on and the AR is phosphorylated by either the AKT (protein kinase B) or the mitogen-activated protein kinase (MAPK) pathway, producing a ligand-independent AR. In the bypass pathway, parallel survival pathways, such as that involving the anti-apoptotic protein BCL2 (B-cell lymphoma 2), prevent the need for AR or its ligand. Compromising AR expression by siRNA induced PI3K-independent activation of Akt, which was triggered by calcium/calmodulin-dependent kinase II (CaMKII). Expression of CaMKII genes is tightly controlled by AR. It dampens CaMKII gene expression whereas abolition of AR activity results in an elevated level of kinase activity and in enhanced expression of CaMKII genes that activate anti-apoptotic PI3K/Akt pathways. Refractoriness to apoptosis is faced as overexpression of CaMKII genes desensitizes cells to apoptosis induced by KN-93, a CaMKII inhibitor, or wortmannin, a PI3K/Akt inhibitor, in terms of combinatorial drug use with doxorubicin, thapsigargin and TRAIL. Moreover, overexpression of CaMKII augments secretion of prostate specific antigen and promotes cell growth of LNCaP in steroid-free condition (illustrated in Fig. 2). There is an integration of two distinct transduction pathways including AR- and CaMKII-mediated pathways. CaMKII is an imperative performer in prostate cancer cells ability to escape apoptosis under androgen ablation and facilitate the progression of prostate cancer cells to an androgen independent state [12, 13].

KN-93 (CaMKII inhibitor) has a broader effect on apoptosis than just inhibition of CaMKII: It inhibits AR activity and induces p53-independent apoptosis, inhibits anti-apoptotic protein Mcl-1, upregulates pro-apoptotic protein PUMA and generates ROS. Phenotype of prostate cancer cells undergoes transition from TRAIL-resistant to -sensitive in combinatorial drug treatment. This is suggestive of the fact that KN-93 could be used for novel therapeutic approaches when hormonal therapy has failed (Rokhlin et al. [11]).

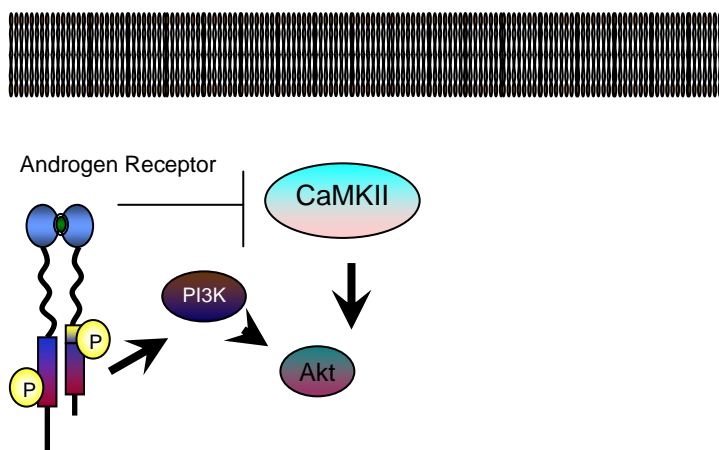


Fig. 2 Androgen receptor suppresses the crosstalk of CaMKII with Akt. However if there is an inhibition of AR, it de-represses CaMKII.

MiRNA: Mini miracles friend or foe

MicroRNAs are non-coding, endogenously synthesized RNAs that adjust gene expression post-transcriptionally. Undeniably plays an imperative role in the execution of carcinogenic pathways and display differential expression in tumor versus corresponding normal tissue and certainly gaining the attention of oncologists. They have decisive task in pathogenomics of the disease and its gain of androgen independency. Significant tools for diagnostic, prognostic and monitoring purposes. Additionally miRNAs might offer exciting avenues for new therapeutic strategies, especially in patients with tumor subtypes that do not respond impressively to currently obtainable therapies. The rapid gain of the attention of the researchers support the hypothesis that miRNA will get an important slot in clinical practice. An overview of current knowledge of miRNA function in prostate cancer will enable oncologists to envision future opportunities and challenges of this research field.

There seems to be an unconquerable state as prostate cancer cells escape apoptosis after androgen depletion or knocking down AR expression and DNA damaging agents are incompetent to activate p53 in the absence of AR and as a result p53 down stream targets especially microRNA-34, cannot be activated. Hampering of AR (si-AR) severely compromised apoptosis, induced by topoisomerase inhibitors doxorubicin (DOX) and camptothecin (Campt). DNA damage inducing agents lead to expression of a variety of apoptosis-related genes including microRNA (miR)-34a and 34b/c following activation of p53. There was a remarkable increase in the expression level of miR-34 increased after DOX, but no increase was found after ablation of AR. It seems logical that AR-dependent inhibition of p53 resulted in suppression of miR-34a and -34c expression. Outstandingly, DOX did not induce miR-34 in LNCaP grown in an androgen lacking medium or in AR-negative prostate cancer cell lines, DU145 and PC3. It is intriguing and worth mentioning that inhibition of miR-34, either 34a or 34c individually, or forced over expression of miR-34a or miR-34c did not induce apoptosis. Only simultaneous inhibition or forced over expression of both miR-34 resulted in modulation of DOX-mediated apoptosis. Collaboration between miR-34a and 34c plays an important role in AR-dependent p53-mediated apoptosis in prostate cancer. This is an unresolved question that has to be taken into consideration to get a step closer to the eradication of the disease. The tight interaction which works so well in diametrically opposed situations is reasonably objectionable and a closer look at the signalling cascades is unavoidable that works so faithfully in “evens” and “odds” [12, 13].

MiR-34a expression was markedly reduced in p53 deficient cells however p53 competent cells were responsive for a robust expression. SIRT1 mRNA and protein levels were decreased after expression of miR-34a. Consistent with the observations it is noteworthy that miR-34a-induced SIRT1 inhibition occurred at the transcriptional but not post-transcriptional level regardless of the presence of a potential miR-34a binding site within its 3'-UTR. miR-34a expression dampens chemoresistance by inducing apoptosis. Henceforth p53 deficient prostate cancer can be addressed utilizing the therapeutic potential of mitrons [2] (illustrated in Fig. 3).

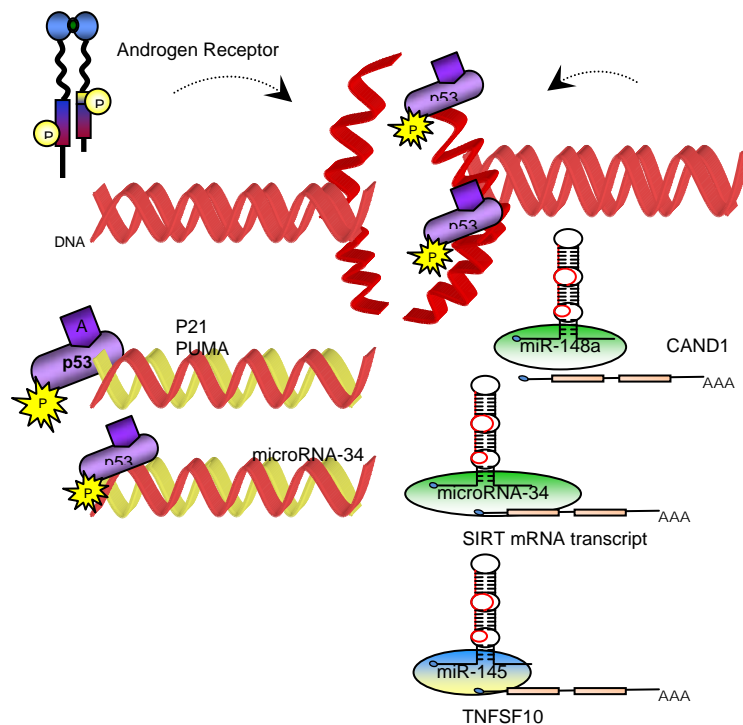


Fig. 3 Double stranded break results in the phosphorylation of p53 that stimulates the expression of miRNA34. This Mitron suppresses the expression of SIRT. Silencing if SIRT enhances the acetylation of p53 and enables it to trigger the expression of p21 and PUMA. MiR-148a silences CAND1 post-transcriptionally. However miR-145 mediates the expression of TNFSF.

MiR-34a regulate silent information regulator 1 (SIRT1) expression through a miR-34a-binding site within the 3' UTR of SIRT1. This inhibition leads to an increase in acetylated p53 and expression of p21 and PUMA. Importantly miR-34a itself is a transcriptional target of p53. It induces expression of miR-34a which suppresses SIRT1, increasing p53 activity [16, 17].

Recent advancements in cancer biology have unmasked involvement of microRNAs (miRNAs) as cancer-related genes. They function as tumor suppressors or oncogenes according to a spatio-temporal pattern. Androgen-dependent gene network is major participant in tumor growth. Murate et al. [13] identified that androgen-responsive miRNAs tethered to the 3'-untranslated region of CAND1 mRNA and down regulated the expression of cullin-associated and neddylation-dissociated 1 (CAND1), a negative regulator of SKP1-Cullin1-F-box (SCF) ubiquitin ligases. Analogously ablation of CAND1 by small interfering RNA promoted the proliferation. It means that this miRNA is solely an oncogene and a therapeutic intervention of this aspect will guarantee a successful outcome. Similar

oncogenic trend is followed by miR-21. It contributes to the resistance of prostate cancer cells to docetaxel and targeting miR-21 may offer a promising therapeutic approach in sensitizing prostate cancer to docetaxel treatment [5]. On the other hand some miRNAs are tumor suppressors and are down regulated in tumor progression. One of the genes significantly upregulated by miR-145 overexpression is the proapoptotic gene TNFSF10. Therefore, modulation of miR-145 may be an important therapeutic approach for the management of prostate cancer as more detailed and clear in-vitro studies of the expression profile of this miRNA can be helpful for disease mitigation [19].

Prostate stem cells: Bolt from the blue

Prostate gland portrays an extraordinary capability to regenerate after successive cycles of castration and retrieval of normal androgen levels. This unbelievable phenomenon points towards existence of a micro-environment of hormone-insensitive cells with stem cell characteristics. The work done by Leong and colleagues, [10] seems to be a reappraisal of the postulates of Isaacs and Coffey [9] who emphasized existence of androgen-independent tissue stem cells in the prostate more than 20 years ago. They observed prostate regeneration after recurring cycles of androgen deprivation and replacement. It is of substantial importance to both basic and clinical scientists [6].

The touchstone of stem cell function is the ability to self-renew, which is a requisite for maintenance of tissue entity over a lifetime. To assess the self-renewal ability of CD117+ cells and to evaluate the frequency of stem cell activity in the CD117+ population Leong et al [7] documented a role for the CD117 antigen in prostate stem cells by an administration of anti-CD117 blocking antibody to castrated mice. They drew a conclusion that knock out of CD117 signaling led to in vivo inhibition of prostate regeneration. In contrast to other cancer types few tools are available for the molecular categorization of prostate cancer. This scarcity restricts personalized treatment approaches for the disease.

Conclusion and future directions

Using state-of-the-art approach, researchers have deeply investigated the vital pathways and machinery that are inter-connected with the anomaly. Findings advocate the presumption that prostate cancer is a multifaceted disease for which simple answers will not be accommodating. Oncologists are beginning to obtain missing links about the critical regulators that may be engaged in the exacerbation of the disease. Truthfully if 1990s was the decade of molecular genetics, which unraveled the paradoxes by sequencing of the human genome, then we now fit well into the era of translational medicine. For oncologists, unfolding the mystery of “tiny miracles” in the prostate will demand a reconsideration of the “prostate particulars”. It must not be overlooked that all prostate cells whether normal or cancerous are not directly sensitive to androgens. Surely insightful approach should be helpful in scrabbling hormone-based strategies for prevention and treatment of prostatic disease.

Consistent with the same concept of clinical strategies, nutraceuticals is another attractive option. These are “natural” substances isolated from food substances and utility is evaluated in a medicinal fashion. Quite a lot of naturally derived food substances have been considered in prostate cancer in an effort to identify natural preventative therapies. Unfortunately; substantial fraction of the literature involving nutraceuticals in prostate cancer is either epidemiological or retrospective. There must be a reassessment of the potential of “natural compounds” in future studies by well-made clinical trials focusing on combinatorial drug design.

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