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Getting Personal with Prostate Cancer: Adding New Pieces to an Incomplete Jigsaw Puzzle

Key Words

Castration-resistant prostate cancer · Androgen deprivation therapy

Abstract

Prostate cancer is a multifaceted molecular anomaly that is insurmountable to date because of the orchestrated network of negative regulators that drive carcinogenesis. A substantial fraction of information has been added that gives yet an unclear snapshot of therapeutic interventions in prostate cancer. Increasing sophisticated interpretations point towards some important aspects of prostate cancer aggressiveness like microRNAs, prostate cancer stem cells and TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) refractoriness. In this review, we will evaluate the push and pull between oncomirs and tumor suppressors in tipping the scales of cancer. Furthermore, multicomponent rational drug designs with a claim to overcome stumbling blocks will be discussed. Translation of the outcomes achieved in the understanding of carcinogenesis at the patient's bedside is possibly the principal challenge in cancer research.

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Introduction

Prostate cancer encompasses a heterogeneous assembly of tumors, with distinctive risk factors, clinical presentation, histopathological features and molecular uniqueness. Currently, cure of metastatic or recurrent disease is dependent on conventional chemotherapy combination regimens. It is a matter of deep concern that efficient clinical management of prostate cancer has been relentlessly challenged by considerable intratumoral multiplicity at genomic and pathological levels and a limited understanding of the genetic elements governing disease development.

Androgen Receptor Dynamics: 'Heads', It Wins, and 'Tails', Therapeutics Lose

Neoplastic cells have to perpetuate the androgen receptor (AR)-mediated dynamics by a wide range of integrated pathways that crosstalk at various levels. It ensures the robust expression and activation of AR-mediated genes. There are multidirectional pathways opted for by the AR to meet the demands of a desperate cancer-prone environment. These pathways have been studied elsewhere [1, 2].

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Fig. 1. AR variants documented by different research groups.

Li et al. [3] registered a robust expression of truncated AR isoforms in 22Rv1 cells along with intragenic reconstitution of an approximately 35-kb AR genomic segment harboring a cluster of alternative AR exons. Cloning of the break fusion junction in 22Rv1 cells displayed long interspersed nuclear elements (LINE-1) encompassing the rearranged segment and a microhomology-mediated break-induced replication. Henceforth, intragenic rearrangements have emerged as new mediators in cancer progression. Some other variants documented by other research groups are mentioned in figure 1 [4–7].

Arachidonic acid pathway members PLA2G7, HPGD, EPHX2 and CYP4F8 were identified as putative novel therapeutic targets in prostate cancer. EPHX2 and PLA2G7 correlated with AR and their inhibition reduced AR signaling. It is obvious that inhibition of these enzymes may be effective when combined with other treatments, such as androgen deprivation [8]. Lately, Kim et al. [9] have found that HOXB13 is a protein that is involved in suppression of AR-mediated signal transduction. This protein is downregulated in prostate carcinogenesis. Future research might converge upon mechanisms of restoration of the negative regulators of AR signaling. There are some other proteins which coexist at the promoter region and are involved in activation of target genes. H2A.Z and ubiquitin-specific protease 10 are each required for transcriptional activation of the AR-regulated prostate-specific antigen and KLK3 genes [10]. A combinatorial drug design seems to dampen the key regulators which augment disease progression. Pharma-

cological targeting of STAT5 retards carcinogenesis because STAT5 cooperates with AR and favors cancer progression and aggressiveness [11]. Yang et al. [12] stated that HDAC4 enhances AR SUMOylation, raising the likelihood that deacetylase may act as an E3 ligase for AR SUMOylation. Knockdown of HDAC4 increases the activity of endogenous AR. Further studies are important to explore antineoplastic detailed mechanistic insights. Another tumor suppressor gene, BTG2, is downregulated in prostate cancer, and the re-establishment of this gene in BTG2-deficient cells has been shown to inhibit prostate cancer cell growth [13]. On a similar note, GLI1, important for Sonic hedgehog signal transduction, can act as a corepressor to considerably inhibit AR-mediated transactivation [14].

Tan et al. [15] stated that treatment of AR by dihydrotestosterone potentially activated GRP78 expression, and there was a coexistence of GRP78 with Hsp70-Hsp90 client proteins. All these proteins limit the efficacy of drugs. A multipronged drug design is necessary to checkmate this group of negative regulators.

Accumulating data mark these AR variants as candidates for therapies directly targeting the AR rather than the ligand. Castration-resistant prostate cancer cells express variant ARs which included truncated ARs lacking the carboxyterminal ligand-binding domain, and small interfering RNA-mediated knockdown efficiently suppressed androgen-independent cell growth. Nigericin-like compounds suppress AR expression at the messenger RNA level. These could be applied as new-type therapeutic agents that inhibit a broad spectrum of AR variants in hormone-refractory prostate cancer [16]. However, there are some compensatory pathways which are switched on impairment of AR. Ablation of AR in in vitro cells induced PI3K-independent activation of Akt, which was triggered by calcium/calmodulin-dependent kinase II (CaMKII) [17, 18]. It has lately been found that grape seed extract potentially inhibits histone acetyl transferase, leading to decreased AR-mediated transcription and cancer cell growth [19]. Similarly, procyanidin B3, an inhibitor of histone acetyltransferase, has broader implications in the inhibition of p300-dependent acetylation of ARs [20].

After having outlined the current approaches for treating prostate cancer, we now focus on emerging therapeutic opportunities for prostate cancer aggressiveness that are based on recent insights into molecular off-track activities of AR. Targeted therapeutics, particularly those that restrain the activity of ARs that are mutated and/or overexpressed in cancer, have revolutionized the treatment. Hsp90-based therapy has so far shown inadequate

activity in the clinic. In accordance with the same interpretation, efficacy of a novel mitochondrial-targeted, small-molecule Hsp90 inhibitor, gamitrinib (GA mitochondrial matrix inhibitor), was evaluated by Kang et al. [21] in the Transgenic Adenocarcinoma of the Mouse Prostate model. They registered that gamitrinib has pre-clinical activity and favorable tolerability in a genetic model of localized and metastatic prostate cancer in immunocompetent mice. Another drug that has wider implications in blocking cancer activity is MDV3100; it blocks ligand receptor engagement and prevents nuclear shuttling of AR and coactivator recruitment of the ligand-receptor architecture [22].

There are an overwhelming number of trials done in an effort to put a stop to cancer aggressiveness, and therefore, one of the foremost goals in the future must be to 'bridge the gap between cancer research and effective therapies'.

Twists and Turns on TRAIL to Therapy

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated therapy has emerged as a breakthrough in translational medicine; however, after accumulation of substantial information into the existing pool of therapeutic interventions, it is obvious that there is a refractoriness to TRAIL treatment.

KN-93 (a CaMKII inhibitor) has a broader effect on apoptosis than just inhibition of CaMKII: KN-93 also synergistically induces cell death in combination with low doses of doxorubicin and converts the phenotype of prostate cancer cells from TRAIL resistant to TRAIL sensitive. This is suggestive of the fact that KN-93 could be used for novel therapeutic approaches when hormonal therapy has failed [23]. TRAIL has been used in conjunction with a broad spectrum of phytonutrients, and results were positive in terms of sensitization of cells to TRAIL [24–26]. These nutraceuticals sensitize cells to TRAIL by upregulating death receptors. In prostate cancer, transforming growth factor (TGF) signal transduction has antineoplastic activities. These activities are attenuated by negative regulators of TGF signaling. We have shown that negative regulators of TGF transduction cascades, especially SMURF and NEDD4, are actively involved in downregulation of TRAIL and upregulation of cFLIP. cFLIP is believed to interfere with the initiation of an apoptotic response by TRAIL. Treatment of cells with TGF and depletion of SMURF and NEDD4 resulted in the upregulation of TRAIL and in the subsequent sup-

pression of cFLIP [27]. Recently, we have documented that abrogation of SMURF and NEDD4 results in activation of ataxia-telangiectasia mutated which negatively regulates cFLIP [28, 29].

MicroRNA: Mini Miracles – Friend or Foe?

An overview of the current knowledge of microRNA (miRNA) function in prostate cancer will enable oncologists to envision future opportunities and challenges of this research field.

miRNAs have broader implications in terms of induction of apoptosis in neoplastic cells. miR-34a and miR-34c play an important role in AR-dependent p53-mediated apoptosis in prostate cancer [16, 17]. miR-34a expression is reduced in p53-deficient cells; however, cells reconstituted for the p53 gene restored the expression of miR-34a [30]. p53 induces the expression of miR-34a which suppresses SIRT1, increasing p53 activity [31, 32].

In a similar way, miR-21 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 [33, 34].

In prostate cancer, evidence has pointed towards the contribution of the androgen-dependent gene network to tumor growth. Murata et al. [35] identified androgen-responsive miRNAs and documented that miR-148a down-regulates the expression of a tumor suppressor, cullin-associated and neddylation-dissociated 1 (CAND1), by binding to the 3'-untranslated region of CAND1 messenger RNA.

On the other hand, some mitrons are tumor suppressors and are downregulated 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 mitron can be helpful for disease mitigation [36].

miR-8, miR-200c and miR-141 being antineoplastic act as potent inhibitors of Notch-induced overgrowth and tumor metastasis [37]. On a similar note, miR-125b and the miR-99 family members miR-99a, miR-99b and miR-100 are tumor suppressors. There is a remarkable downregulation of these mitrons in prostate cancer progression. On the other hand, restoration of these mitrons resulted in the reduction in expression of prostate-specific antigen. SMARCA5 and SMARCD1 and the growth regulatory kinase mTOR are triggered by the miR-99 family [38].

Fig. 2. An overview of miRNA processings and implications. NF- κ B = Nuclear factor- κ B; FSCN = fascin homolog; MnSOD = manganese superoxide dismutase; GPX2 = glutathione peroxidase 2; TrxR2 = thioredoxin reductase 2.

The miR-17-92 cluster has broader involvement in terms of suppression of mitochondrial antioxidant enzymes, such as manganese superoxide dismutase, glutathione peroxidase 2 and thioredoxin reductase 2. It is documented by Xu et al. [39] that miR-17* binding sequences are located in the 3'-untranslated regions of these 3 target genes. Transient transfection of neoplastic cells with miR-143 and miR-203 resulted in regression of proliferation and invasive potential [40, 41] (fig. 2).

Existing polymorphisms in miRNAs and respective target sites underpin variant clinical outcomes in prostate cancer patients [42].

miR-221 and miR-222 are oncomirs which are engaged in the suppression of tumor suppressor genes. miR-221 and miR-222 inhibitors caused significant induction of a tumor suppressor aplasia Ras homolog member I (ARHI). A direct interaction of miR-221 or miR-222 with a target site on the 3'-UTR of ARHI was confirmed by Chen et al. [14] in 2011. It is also worth mentioning that genistein upregulates ARHI by attenuating miR-221 and miR-222 in prostate cancer cells.

Mounting evidence indicates that some proteins are involved in the stimulation of mitrons. Nuclear factor- κ B triggers miR-221/222 expression in prostate cancer. Recently, Galardi et al. [43] identified two regions upstream of the miR-221/222 promoter which are bound by the nuclear factor- κ B subunit p65.

While drawing a parallel between advanced-stage patients and localized disease patients, levels of miR-21, miR-141 and miR-221 were significantly higher in advanced stages than in patients with localized disease [44]. It is also interesting to note that a relatively higher level of miR-100 is related to biochemical recurrence of localized prostate cancer in patients treated with radical prostatectomy [45].

Reconstituted cells for miR-145 displayed an inhibited cell proliferation, migration and invasion. This antineoplastic activity was modulated by a significantly decreased signal at two miR-145 target sites at the 3'-UTR of fascin homolog 1, suggestive of the fact that miR-145 negatively regulates fascin homolog 1, an actin-bundling protein [46].

Keeping in view the crests and troughs between methylation and loss of imprinting, it is documented by Hulf et al. [47] that miR-205, miR-21 and miR-196b are epigenetically repressed, and miR-615 is epigenetically activated in prostate cancer cells.

miR-34a is a p53 target and was underexpressed in CD44(+) prostate cancer cells. CD44 knockdown was similar to miR-34a overexpression in retarding prostate cancer metastasis [48].

Phytonutrients stimulate the expression of tumor suppressors. Siddiqui et al. [49] observed a suppression of androgen-regulated miR-21 and upregulation of a tumor

suppressor, miR-330, in tumors of mice treated with epigallocatechin gallate. miRNAs in tumor microenvironment act in a new network that reinforces tumor suppressor gene expression, reducing the expression of AR-mediated transcriptional activities and activators.

Prostate Stem Cells: Same Ballpark, Different Players and Different Rules

Cells within a tumor are structured and controlled in a hierarchical lineage relationship and portray differential tumorigenic potential, signifying that targeted therapeutics should converge upon rare cancer stem cells that maintain tumor malignancy. In the upcoming section, we will discuss the existing status of studies to identify cancer stem cells in human prostate cancer as well as in mouse models, with an emphasis on discussing different functional assays and relative hopes and pitfalls.

Rajasekhar et al. [50] identified a minor subset of stem-like human prostate tumor-initiating cells (TICs) that neither expressed AR nor prostate-specific antigen. These TICs have stem cell-like hallmark features and multipotency as verified by sphere formation in various in vitro experimentations and in vivo tumor initiation, respectively. The cells give a snapshot of an undifferentiated subtype of basal cells which might be purified from prostate tumors, coexpressing human pluripotent stem cell marker TRA-1-60 with CD151 and CD166. It is worth mentioning that such triple-marker-positive TICs restore the original parent tumor heterogeneity in serial xenotransplantations, underscoring a tumor cell hierarchy in carcinogenesis. It is also interesting to note that the CD44(+) cell population is presumed to have stem cell-like properties. In accordance with this assumption, Yu et al. [51] introduced these CD44(+) and high-activity aldehyde dehydrogenase-containing cells into severe combined immune-defi-

cient mice to determine the corresponding tumorigenic capacities. The cells were able to reinforce tumorigenesis in the mice. Additionally, Yin et al. [52] isolated the cancer stem cell-like side population cells from the human prostate cancer cell line DU-145 by a flow cytometry-based side population technique. The research group was able to characterize various proteins upregulated in prostate cancer stem cells. Notably, arachidonate 12-lipoxygenase and ATP-binding cassette subfamily G member 2 are upregulated in prostate cancer stem cells. Lately, Rybak et al. [53] reported enrichment and characterization of sphere-propagating cells with stem-like properties from DU-145 prostate cancer cells in a defined serum-free medium.

Another important breakthrough in prostate cancer stem cell initiation was given by Chang et al. [54] who stated that Sonic hedgehog signal transduction is involved in transforming normal prostate basal/stem cells into prostate cancer stem cells. Similarly, Nodal, a TGF- β -like growth factor, maintains the pluripotency of embryonic stem cells. Nodal mediated proliferation and cellular migration via ALK4 and ActRII/ActRIIB receptors and Smad 2/3 phosphorylation. It clearly indicated that Nodal-mediated signaling cascade is enhanced in cellular transformation [55].

Conclusion and Future Directions

Prostate cancer exempts a large number of therapeutic tools, yet many leading methodologies stand in queue. Being a multifactorial anomaly, prostate cancer overtakes a complex of interrelated molecules. miRNAs and stem cells occupy the top slot. All these factors are substantially intertwined, so mono-oriented targeting is of no use. Making up with these new parameters may give a sharp optimistic twist to future interventions in prostate therapeutics.

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