Systems Medicine Approaches to Improving Understanding, Treatment, and Clinical Management of Neuroendocrine Prostate Cancer

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Abstract: Background: Prostate cancer is the most commonly diagnosed cancer in men. More than 200,000 new cases are added each year in the US, translating to a lifetime risk of 1 in 7 men. Neuroendocrine prostate cancer (NEPC) is an aggressive and treatment-resistant form of prostate cancer. A subset of patients treated with aggressive androgen deprivation therapy (ADT) present with NEPC. Patients with NEPC have a reduced 5-year overall survival rate of 12.6%. Knowledge integration from genetic, epigenetic, biochemical and therapeutic studies suggests NEPC as an indicative mechanism of resistance development to various forms of therapy.

Methods: In this perspective, we discuss various experimental, computational and risk prediction methodologies that can be utilized to identify novel therapies against NEPC. We reviewed literature from PubMed and computationally analyzed publicly available genomics data to present different possibilities for developing systems medicine based therapeutic and curative models to understand and target prostate cancer and NEPC.

Results: We discuss strategies including gene-set analyses, network analyses, genomics and phenomics aided drug development, microRNA and peptide-based therapeutics, pathway modeling, drug reppositioning and cancer immunotherapies. We also discuss the application of cancer risk estimations and mining of electronic medical records to develop personalized risk predictions models for NEPC. Preemptive stratification of patients who are at risk of evolving NEPC phenotypes using predictive models could also help to design and deliver better therapies.

Conclusion: Collectively, understanding the mechanism of NEPC evolution from prostate cancer using systems biology approaches would help in devising better treatment strategies and is critical and unmet clinical need.

Keywords: Prostate cancer, precision medicine, systems biology, neuroendocrine prostate cancer.

INTRODUCTION

An estimated 2.9 million people are living with prostate cancer (PCa) in the US. Nearly 27,500 patients will die this year due to complications arising from the disease, making it the second leading cause of cancer-related deaths in American men [1]. The SEER database also suggests PCa to be the most commonly diagnosed cancer in American men; more than 220,000 new cases diagnosed each year with a lifetime risk of 1 in 7. Localized prostate cancer is usually treated by either surgical excision (Radical Prostatectomy) and/or radiotherapy, both of which cure a majority of the patients. However, in a small proportion, recurrence and metastasis develop. The metastatic disease is routinely treated using androgen deprivation therapy (ADT) but invariably progresses to a castration-resistant prostate cancer (CRPC) phase with poor prognosis. Recently approved AR-signaling inhibitors Enzalutamide and Abiraterone have been shown to be effective in more than 50% of the CRPCs [2-7]. However, 50% of the responders develop resistance to the new treatments within two years [8-11]. Analysis of tumors from these patients suggests that nearly 25% of them present with an aggressive and treatment resistant form of the disease called neuroendocrine prostate cancer (NEPC) [12, 13]. There is a recent interest in understanding the mechanism of differentiation of CRPC to a neuroendocrine form and developing better diagnostic and precision therapies to manage these lethal and drug resistant tumors. In this comprehensive perspective, we discuss the cancer biology of NEPC, various strategies to identify potential biomarkers of NEPC, opportunities to characterize potential drug targets and develop precision therapy using systems medicine-based approaches.

NEUROENDOCRINE PROSTATE CANCER

The prostate parenchyma is made up of prostatic acini surrounded by stromal, fibroblast, nerve and endothelial tissues. The acini are comprised of basal and luminal cells and few interspersed neuroendocrine cells around the basal cells that are AR-negative (Fig. 1). These cells secrete neuropeptides such as bombesin, neutorphin, serotonin and calcitonin that have been postulated to support the growth of both normal and malignant prostate tissues [13, 14]. The diagnosis and sub-classification of NEPC is principally carried out using the evidence gathered from cellular morphology, distribu-
tion and expression of well-known neuronal markers, such as neuron-specific enolase (NSE, expressed from ENO2), chromogranins (CHGA/CHGB), synaptophysin (SYP) and CD56 [15, 16]. NEPC can be divided into five subclasses: a) adenocarcinomas with focal NE differentiation, b) adenocarcinomas with Paneth-cell-like NE differentiation, c) carcinoid tumors d) large cell carcinoma and e) small carcinoma. Of these, small cell carcinomas are the most common representative of the cellular phenotype [17].

The percentage of neuroendocrine (NE) cells has been shown to increase with disease aggressiveness, suggesting their role in either supporting the growth of cancer cells or bringing about resistance to therapy. Whereas PCa primarily arises primarily from the acinar cells [18], newly diagnosed NEPC, which comprises less than 1-2% of all prostate cancers, arise from the NE cells [17]. The concomitant increase in the NE cells with aggressive disease progression has been purported as a mechanism of resistance development, and termed as treatment induced neuroendocrine prostate cancer (tNEPC) [14, 19]. Indeed in vitro studies with PCa cell lines suggest NEPC development as a mode of resistance to ADT and radiation therapy [20-22].

In the human phenotype ontology, prostate neoplasm and endocrine neoplasm are described using separate term hierarchies (Fig. 2) and the transition of PCa phenotype to NEPC as part of the chemotherapy resistance mechanism combines the pathological features of both phenotypes. In the tumor evolution setting (where ADENOCARCINOMA \(\rightarrow\) CRPC \(\rightarrow\) NEPC; Figs. 1 & 3) a series of changes are brought about not only to promote the tumor proliferation and migration to visceral organs, but also to initiate signaling alterations that drive neuronal gene expression [23, 24].

**NEURONAL CORRELATIONS OF PROSTATE CANCER**

Several lines of evidence indicate a high correlation between neuronal structures and prostate cancer. PCa involved with perineural invasion show more aggressive and proliferative phenotype (Fig. 3) [25]. Studies in mice models suggest the involvement of the autonomic nervous system in the initiation (sympathetic nervous system) and progression (parasympathetic system) of PCa [26]. Furthermore, epidemiological studies have identified an inverse correlation between PCa and nervous system disorders such as patients with spinal cord disorders have small prostate sizes and lower chances of developing PCa [27], and patients with central nervous system (CNS) diseases including schizophrenia, Parkinson’s and Alzheimer’s disease have a lower likelihood of developing PCa [28]. Finally, interleukin-6 (IL6) not only plays a significant role in PCa progression and NE differentiation [29-32] but also has a pathological role in CNS disorder schizophrenia [33-35]. Thus, emerging evidence suggests that PCa has a predisposition towards developing into a neuronal phenotype due to the shared signaling and genetic cues and may explain the observation of anti-psychosis drugs having a suppressive effect on PCa development [36, 37].

**EXPERIMENTAL MODEL FOR NEPC**

To understand the processes underlying the differentiation of ADENOCARCINOMA \(\rightarrow\) CRPC \(\rightarrow\) NEPC, in vitro models using prostate cell-lines have been developed. Upon transformation, the cells display dramatic morphological changes associated with neurons such as branched neurite formation and expression of neuronal markers (Fig. 2). One of the first such studies identified cAMP as a ligand that brings about differentiation of LNCaP (an AR responsive adenocarcinoma cell line model derived from lymph node metastasized PCa) cells in vitro [38]. Another study identified androgen deprivation leads to neuronal differentiation of LNCaP cells as well [39, 40]. Thereafter, several other studies demonstrated the use of other factors such as IL6 [41], neuropeptide bombesin [42], WNT-11 [43], radiation [22], valporic acid [44], genistein [45], vasoactive intestinal peptides [46, 47], Jolkilonide B [48], and COX-2 inhibitor [49] to bring about NE differentiation. However, most of these studies have been limited to using the LNCaP adenocarcinoma cell line model, which does not reflect clinical observation of ADENOCARCINOMA \(\rightarrow\) CRPC \(\rightarrow\) NEPC. A ligand capable of converting prostate cancer cell lines in to the NE form, irrespective of AR status, would be crucial in understanding the progressive mechanism of prostate tumor evolving towards a neuroendocrine phenotype. Unfortunately, the in vitro transformed cells undergo post-mitotic arrest preventing their use as a model system to test drug efficacy studies.

The poor availability of a purely NEPC cell line has limited drug screening and testing efforts. The PC3 cells (an AR negative CRPC line derived from a bone metastasized PCa) have been shown to share characteristics of NEPC and express markers such as NSE and Chromogranin [50]. However, the NCI-H660 cell-line, which was initially attributed as a small cell lung cancer cell line [51], and later confirmed to be of prostate origin using cytogenetic studies [52], exhibits all characteristics of NEPC. It also harbors the prominent TMPRSS2-ERG fusion that is present in more than 50% of all prostate cancers as well as a mutated form of TP53 [53, 54]. Importantly, genomic and biochemical studies have identified it to be a suitable model of NEPC, expressing all the neuronal markers, and has been successfully utilized to test small-molecule drug against NEPC [23].
SYSTEMS MEDICINE APPROACHES FOR TARGETING NEPC

Systems medicine is defined as an interdisciplinary approach to study a disease at the systems level of the human body by considering it as part of an integrated complex system with biochemical, physiological, and environmental interactions [55]. NEPC is treated primarily with chemotherapy, especially a combination of docetaxel with etoposide and cisplatin. However, resistance development is very rapid, and survival is less than 1.5 years [56, 57]. Pre-clinical success with zoledronic acid [58], plumbagin [59] and danusertib [23] has also been reported. The prevalence of high-throughput sequencing and phenotyping is revolutionizing design, development and delivery of precision medicine for various cancers including prostate [60]. Kidd et al. illustrated an example of a case-study on how data-driven precision therapy could guide in designing patient-specific therapy [61]. While personalized molecular profiling and individualized treatment strategies have conflicting outcome reports [62]—personalized, data-driven recommendation and its integration with predictive models could provide a better outcome for patients with NEPC.

THERAPEUTIC STRATEGIES TO TARGET NEPC

Designing experimental and computational strategies for the systematic targeting of pathways, genes or genetic variations involved in PCa or NEPC could help to find better therapies for NEPC. Such methods include gene-set-based target discovery, network medicine approaches, genotype-phenotype aided drug discovery, development of protein or peptide drugs, miRNA-informed therapy, targeting gene or transcript-fusion events, pathway-based drug development, high-throughput screening of small molecules and drug repositioning approaches.

MOLECULAR ARCHITECTURE OF PCA AND NEPC

Multiple gene sets are currently available to perform systematic analyses of PCa and NEPC. From the first report in the late ’90s on the role of BMX (Etk/BMx) in NEPC [41] till 2015 more than 30 additional genes, such as DEK [63], PEG10 [64], CREB, IL-6 [32, 65-67], EGF [68], ASH-1 [69] Midkine [70], Mash1 expression [71], AKT/hnRNPK/AR/beta-catenin [72], MmSOD [73], Wnt-11 [43], SNAIL1 transcription factor [74], SOD2 [75], CD44 [76], NeuroD1 [77], Cystatin C [78], IL8 [79], Granin A and Calcitonin [80], Ps2 protein [80], BCL-2 [81]), and their expression pattern have been studied using in vitro and in vivo model systems. Next generation studies (NGS) over the last few years, including the recent NGS-report from the TCGA, have provided extensive catalog of genes and subtypes associated with PCa and its subtypes including NEPC [20, 23, 82, 83]. Observation of factors previously identified in adenocarcinomas (such as TMPRSS2-ERG translocation [84]), strongly suggests the progressive development of NEPC from adenocarcinomas through the CRPC phase [85]. Specific oncogenic drivers (such as MYCN [86, 87], MYCC [88], PLK1 [89], and AURKA [23] are activated, and tumor suppressors such as TP53 [90], RB1 [91], along with transcription factor, RE1-Silencing Transcription Factor (REST1, alias Neural-Restrictive Silencing Factor [24]), are lost or downregulated. Upregulation of epigenetic regulators such as EZH2, H2S/Cav3.2 [92], Secretogranin II [93], Orexin type 1 [94], AGR2 [95] have also been identified as key players of NEPC pathology. Selective inhibition of driver genes, such as AURKA, has been shown to alleviate the NEPC phenotype [23]. Several canonical gene sets are reported in the literature indicating involvement in NEPC and pleiotropic mechanisms where one gene influences multiple traits have been implicated in PCa [96]. Cancer sub-type specific gene-sets from whole-exome, genome and transcriptome studies are reported as part of TCGA studies. A re-
cent study also reported the expression quantitative trait loci (eQTLs) associated with genetic variants involved in PCA [97]. Such lists of genes, transcripts and proteins associated with PCA and NEPC are continuously expanding. Thus, designing drugs that can reverse the validated expression signature could aid in developing new therapies for NEPC.

NETWORK ANALYSES OF GENE-SETS TO FIND KEY HUBS DRIVING CANCER PATHOLOGIES

Network analyses of the candidate, or curated genes discovered from NGS profiling can be used to not only find driver and passenger genes but also genes that are vital to the disease pathways [98]. Deriving network analyses based connectivity metrics of PCA or NEPC genes can be useful to pinpoint key players of pathophysiological mechanisms. Understanding these fundamental mechanisms could help to identify new targets and new therapies. A recent study explored the key proteins associated with ovarian cancer by converging nanobiotechnology and network analyses [99]. Briefly, nanoparticles were used as therapeutic agents to quench the growth of ovarian cancer cell lines. Using gold-nanoparticle tagging and mass spectrometry, the proteins involved at various progressive phases were identified. The proteins were then used to derive putative functional networks. These networks were then prioritized using connectivity indices like degree, radiality and eccentricity. Developing similar network driven approaches using canonical gene-lists or patient-derived gene sets and combining clinical and biological relevance would help to find key hubs that drive aggressive cancer pathologies of the prostate.

Using a similar computation method, we used the NEPC gene sets from a recent study [23] and compiled a canonical network (Fig. 4). This network can be further perturbed using network metrics and synthetic lethality [100] to find key hubs.

GWAS (GENOME-WIDE ASSOCIATION STUDIES) AND PhEWS (PHENOME-WIDE ASSOCIATION STUDIES)-DRIVEN DRUG DISCOVERY

Whereas GWAS studies aim to find genetic variants associated with a given phenotype using large case and control cohorts, PhEWS is a unique analysis that aims to find the enriched variants associated with genetic variants of a primary phenotype such as phenotypes associated with PCA. PhEWS is generally possible when GWAS patient cohorts are derived from electronic medical record (EMR)-linked biorepositories. GWAS studies have identified several genetic variants and genes associated with PCA, albeit with low risk penetration. Analyzing the compendium of genetic variants, genes and the phenotypes enriched across patients harboring risk variants could help to develop better therapies. The high-quality data captured during the clinical trial or clinical operations are in the silos of clinical registries or internal databases of healthcare or pharma organizations and not readily available for data mining. Current PhEWS are leveraging EMR data as the primary data source that provides the breadth of the phenotypes suitable for high-throughput human phenomics studies.

Genotypes and Genes Associated with PCa

GWAS data is currently being explored to find new targets for a variety of diseases including cancers and autoimmune disease [101]. While there is no phenotype specific GWAS on NEPC, a total of 73 genetic variants have been identified from GWAS in case-control populations of different ancestries with PCA (Fig. 5). These genetic variants were reported or mapped to 511 genes. While a majority of these genes have known biochemical or computationally derived functional roles and 7.4% of these genes are orphan genes with no validated functional or biochemical roles. Exploring the functional or regulatory role of these genetic variants and genes [102] in NEPC could aid in developing GWAS-aided therapies for NEPC patient population.

Phenomic Correlations of Patients with PCA Genotypes

To understand the phenotype-genotype correlation of PCA and other clinical phenotypes, we have compiled the phenotype-wide association datasets from PhEWS database (https://phecwas.mc.vanderbilt.edu/). We compiled 73 variants associated with PCA and their associations with 1218 phenotypes. Peyronie’s disease was the most significant association but with a small number of sample size (n=44, rs2735839, P=4.1E-06; See Fig. 6). This association would need further investigation as Peyronie’s disease is an outcome associated with men that have undergone radical prostatectomy. The genetic variant rs2735839 is an intronic variant of KLK3, a crucial prostate cancer gene and may play a mechanistic role in NEPC. The phenotype “adverse effects of antihypertensives” was the phenotype with the lowest odds ratio (n=56, rs10187424, P=4.1E-05, OR=0.3765). The phenotype “cardiac conduction disorders” had a lower risk in patients harboring variants for PCA (n=2685, rs1983891, P=0.0008882, OR=0.8627). The variant rs1983891 is localized to the intrinsic region of FOXPI, which negatively regulate PCA [103]. Hepatic cancer was the phenotype with highest odds ratio among the 1645 tested phenotypes (n=30, rs7837688, P=0.0001333). The variant rs7837688 is localized to intergenic regions with CASC8 and LOC105377534; and proximal to MYC, a known PCA and NEPC gene. Intronic variants identified from GWAS have been implicated in driving pathological mechanism in various diseases including myocardial infarction [104, 105], and exploring the expression, function and regulatory mechanisms of these variants or their eQTLs may lead to discovery of new mechanisms underlying PCa and NEPC.

PROTEIN AND PEPTIDE DRUGS FOR NEPC

Protein drugs are evolving as a competing therapeutic domain to improve or augment small-molecule based therapies. Peptides
have been shown to be effective in inhibiting previously ‘undruggable’ target such as Ras [106] and transcription factors such as NOTCH [107]. NEPC has phenotypes regulated by peptides and developing specific protein or peptide drugs to modulate these peptides could be of high therapeutic value. For example, NEPC related peptides promote IGF-1R signaling and promote NEPC [108]. Neuropeptides 26RFa [109] and Manserin [110] were implicated in NEPC. Midkine a heparin binding growth factor [70], recombinant human IFN-beta [111] and vasoactive intestinal peptide (VIP) [112] have been shown to have a role in NEPC phenotype development and growth. Inhibiting these peptides using stable peptidomimetics could aid in finding therapies for NEPC.

TARGETING FUSION TRANSCRIPTS ASSOCIATED WITH NEPC

Chromosomal translocations are regarded as a cytogenetic feature of various tumors including PCa [113]. NGS technologies have revealed a large number of fusion genes or fusion transcripts and their putative role in diseases including various cancers, cancer resistance and relapse [114]. For example TMPRSS2-ERG, SLC45A3-BRAF, SLC45A3-ELK4 and ESRP1-RAF1 fusion transcripts have been implicated in PCa [115-117]. Targeting fusion transcripts could pave way towards development of better therapy, similar to strategies utilized to target BCR-Abl with imatinib for CML (chronic myelogenous leukemia) [118].
MicroRNA-BASED THERAPY FOR NEPC

Micro RNAs (miRNAs) are non-coding RNAs that regulate expression of genes post-transcriptionally. Regulation of several androgen-induced genes has been observed by repressing three miRNAs: hsa-mir-125b-1, hsa-mir-125b-2 and hsa-mir-99a [119]. In another study, dysregulation of hsa-mir-145, which targets and regulates expression of ERG [120], and, hsa-mir-183, which targets DKK3 and SMAD4, have been implicated in PCa [121]. Collectively these microRNAs inhibit a set of genes (PRNP, S1PR1, TACSTD2, NR3C1, SOD2, WDR12, CDNF, BMPR2, COX20, IGF1R, ART4, PLEKHA1, AMOTL1, DAZAP2, BMPR2, GT1, BCL2L1, GMFB, BTG2, RPL9, UBN2, TNRC6C, LHFPL2, VAV3, HNRNPA1, ZNF529, WEE1, PTMA, SLC38A1). While a specific role or function of miRNAs in NEPC has not been elucidated, performing miRNA sequencing and analyses of downstream targets could help in the identification of new therapeutic targets for NEPC.

PATHWAY-DRIVEN DRUG DISCOVERY TO TARGET PATHWAY CROSS-TALKS IN NEPC

 Biological pathways act in concert to perform a variety of functions in the “normal” and “disease” states [122]. Emerging evidence from systems-biology studies suggests that pathway cross-talks plays an important role in imparting pathophysiology of multiple diseases and the switch between normal and disease states can be modulated through pathways with regulatory activities [123, 124]. Such approaches can use biological pathway evidences, including pathway cross-talk, and can help in the identification of new indications for existing compounds [125]. There are multiple computational experimental strategies to find new pathway modulators or targets from pathways. For example, vascular endothelial growth factor signaling pathway is targeted by a diverse set of drugs (n=67; see: http://www.genome.jp/dbget-bin/www_bget?pathway+hsa04370) (VEGF, See Fig. 7). VEGF is a critical pathway composed of 61 genes and six organic compounds [126]. VEGF has also been implicated to play a key role in NEPC [127]. Drugs targeting VEGF have a variety of activities including analgesic, anti-inflammatory, anti-neoplastic and anti-rheumatic. Specific disease phenotypes associated with drugs targeting VEGF pathway includes solid tumors, atherosclerosis, neuropathic pain, Acute respiratory distress syndrome (ARDS), major depressive disorder, acute coronary syndrome, Crohn’s disease, Psoriasis and age-related macular degeneration. VEGF is an example of a pathway that can be activated or inhibited by multiple compounds for therapeutic interventions. Identification of similar pathways and their cross-talks using systems biology approaches including quantitative modeling and pathway analytics could help to reposition drugs for new indications [128-136]. Pathways such as Notch signaling [137] and cAMP signaling [138] have established role in NEPC and pathway-based drug development can be used to improve available therapies that can potentially target NEPC.

Targeting Signal Transduction Events of NEPC

Biochemical modulation of pathways or disease mechanisms by inhibition or activation of post-translational modifications including phosphorylation events can act as therapeutic strategies for NEPC. Phosphorylation of protein kinase A by RhoA is reported as a signaling event associated with NEPC [139]. Hormone signaling by paracrine factors [140] has been implicated in NEPC, although the precise roles of these modulations are yet to be ascertained. Inflammation, immune response and activation of macrophages [141] have also been indicated as a mechanistic basis of NEPC. Understanding key modifiers of proteins and pathways of PCa and their role in recurrence is key to understand signaling pathways driving the progression of PCa to NEPC.

Metabolic Pathway Modeling to Identify Drug Targets for NEPC

Targeting metabolic pathways by leveraging computational modeling and simulation offers an effective method to identify novel or better drug targets [142-144]. Computational methods, that can model metabolic pathways, simulate enzyme kinetic activities and perform in-silico gene knockouts, could help in identification and prioritization of new drug targets [145]. Modeling techniques...
like flux balance analysis [146] and its derivatives use genome scale reconstructions of metabolic models to predict and prioritize the role of single, pair or multiple genes using gene or metabolic reaction perturbations. Briefly, the method involves five steps: 1) construction of a draft model using automated genome annotation pipelines, 2) refinement of draft models by leveraging high quality annotations using bio-curation, 3) generation of a computable model and definition of model constrains and parameters for simulations, 4) evaluation of model and simulation of gene or reaction knockout experiments, 5) interpretation of results and experimental validation.

Genome-scale metabolic models for target prioritization can be enhanced by integrating data from transcriptomics, metabolomics, proteomics, biochemical studies and predictive algorithms [147-152]. A list of software packages (See: http://sbml.org/SBML_Software_Guide) and detailed protocols for performing genome-scale reconstruction and modeling is available here [153, 154]. Metabolic modeling has been used to identify drug targets for infectious diseases (tuberculosis [155] and multidrug-resistant Staphylococcus aureus [156]), cardiovascular diseases [157-159], neurodegenerative disease [160], cancer [161], diabetes, and obesity [162]. Metabolic models have also been employed to predict biochemical or physiological response including drug toxicity evaluations as part of drug discovery investigations [163].

HIGH-THROUGHPUT SCREENING TO FIND COMPOUNDS TARGETING NEPC

Target based drug discovery relies on experimental screening of the compounds using a large number of compounds from custom or commercial ligand libraries. The compounds are tested using high-throughput screening (HTS) [164] platforms that enable rapid testing of a large collection of compounds and their agonistic or antagonistic perturbations using cell line based screening assays or specific protein-ligand binding assays (LBA) [165]. Such experimental approaches can be optimized using computational screening method that simulate the screening using virtual-ligand screening method [166]. Virtual ligand screening methods help to reduce the number of compounds that need experimental evaluation and thus help in optimizing the cost of drug discovery investigations. Briefly, the virtual ligand screening method comprise of protein modeling, ligand modeling and structure optimization, protein-ligand docking and assessment of docked complexes using a variety of chemoinformatic parameters [167-170]. Compounds can be assessed using Quantitative Structure-Activity Relationships (QSAR) [171] that provides a quantitative inference of how well the ligand can perturb the target, pharmacophore properties, adsorption, distribution, metabolism and excretion and toxicity (ADMET) profiling and other chemoinformatic parameters that are relevant to identifying the optimal ligand for a given target based on disease biology, physicochemical properties and binding kinetics. Computational evaluation of drug-target interactions would help in focusing downstream functional studies and clinical trials on a subset of compounds and thus help in improving the process of drug discovery by reducing the cost and time to translate therapies from bench to the clinical setting [172, 173].

DRUG REPOSITIONING FOR NEPC

Drug repositioning comprises the use of computational and experimental analyses to identify the suitability of an existing, approved or investigational compound for a new indication. This approach has been used as a therapeutic strategy for more than 250 drugs and spans multiple indications from all classes of diseases.
CANCER IMMUNOTHERAPY FOR NEPC

Cancer immunotherapy is the emerging field of research to design and develop therapeutic interventions, which can activate the immune system for recognition and killing of cancer cells. Immunotherapy was shown to be effective for complex diseases including asthma and melanoma [185-188]. Immunotherapy for cancer comprises of the various strategies employed to either activate the immune effector functions (both innate as well as adaptive) or antagonizing immune inhibitory functions, or both [189-192]. Examples of strategies for activating immune effector functions include adoptive cell therapy (ACT), which introduces immune cells directly into the patient [193, 194], vaccination with tumor antigens [195], cytokine treatment (such as IL-2) [196], induction of antigen presentation (dendritic cell pulsing and administration) [197] and antibodies targeting co-stimulatory molecules for T-cell activation (OX40) [198]. Examples of inhibitory strategies include antibodies that deplete regulatory T cells (anti-CD25) [199] and antibodies targeting immune checkpoints (cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death-1(PD-1) [200]. Several of the above strategies that are in various phases of clinical trials for PCa include; Sipuleucel-T which is a dendritic cell-based vaccine [201, 202], Ipilimumab a fully humanized anti-CTLA-4 monoclonal antibody based immunotherapy [201, 203], Prostavacin-VF (also known as PSA-TRICOM) an attenuated recombinant Vaccinica/Fowlpox viral-vector based vaccine [202] and GVAX, which is a PCa cell-line based vaccine [204, 205].

PCa and NEPC is ideally suited for immunotherapy as it has a long natural history, thus providing ample time for immune effector functions to develop and act against the tumor. It is also rich in several neo-antigens such as PSA and PSMA, thereby providing an excellent repertoire of antigens for targeted immunotherapy. Importantly, the above ongoing-clinical studies suggest immunotherapies to be more efficacious when carried out at an earlier stage where tumor burden is low. Thus, vaccination either alone or in combination with androgen deprivation therapy (ADT) or radiotherapy in pre-surgical disease settings is essential as it will serve three important purposes; 1) define true response criteria to immunotherapies which generally elicit slower responses, 2) help identify markers of disease progression by providing an opportunity to sample tumor and blood both before and after treatment, and 3) provide an oppor-
tunity to identify neo-antigens which could be harnessed for personalized immunotherapy regimes. Although no immunotherapy-based clinical trials are currently focused on NEPC, the advent of new genomics-based-computational prowess that can predict, map and reposition drug that can modulate the immune system holds immense promise in the field [206]. In the future, such systems-based approaches and clinical trials of vaccines in pre-surgical settings would pave the ways towards designing immunotherapies targeting NEPC.

PREDICTIVE STRATEGIES FOR NEPC PATIENT STRATIFICATION

Therapeutic interventions are important in cancer management to provide positive patient outcomes including improvement in the quality of life of patient and reduction of symptom burden. NEPC is primarily a treatment induced, aggressive form of cancer that occur in a subset of patients with poor response to treatment. In this scenario, developing predictive models by combining deep profiling data from tumors and pharmacogenes with phenomic data could aid in developing risk models to see which patients are at risk for NEPC. Identifying the variable to develop such predictive models often need large-scale clinical trials. With the advent of automated data mining techniques and phenotyping algorithms, this is changing. Algorithms can be designed to precisely phenotype PCa or NEPC and these algorithms can be used to compile a large patient population retrospectively. Data can also be assembled from cancer registries and public biomedical and healthcare databases. There are multiple ways to develop such predictive models; here we highlight two cases using EMR mining and risk model development.

MINING OF EHR AND CLINICAL TRIAL DATABASES TO FIND PCa AND NEPC TRAJECTORIES

Systematic analyses of data generated as part of the care-delivery of PCa or NEPC patient populations using data compiled in electronic health records (EHR) would also help to understand clinical implications of NEPC and the evolution of patients from PCa to NEPC or how the patients responds to NEPC treatments. EHR based data mining and digital epidemiology studies are cost-effective, efficient and provide clinical insights more quickly than clinical trials. EHR-linked biorepositories have been used to do phenomics, phenome-wide enrichment analyses and discovering genetic variants associated red blood cell traits, platelet traits and diseases including peripheral arterial diseases [207-210]. Mining multiple data types from EHR has shown to be an important way to find patient clusters that can be treated with an intervention or therapy. For example, Li et al. (See: http://stm.sciencemag.org/content/7/311/311ra174)) have shown that utilizing clinical characteristics including patient demographics, lab measures, and drug/s taken could help to segregate Type-2 diabetes population as three subtypes. Individualized or population scale therapies can now be designed to target these subtypes and improve outcomes. Similar approaches can be adapted for PCa and NEPC cohort and could help to determine patient clusters based on genetic variation, phenotypes or treatment response rates.

RISK PREDICTION MODELS FOR RESPONSE TO PROSTATE CANCER TREATMENT AND NEPC

Developing predictive models to assess the treatment responses (no response, partial response or full response) will be a valuable clinical aid in managing PCa patients and understanding the demographics of NEPC patient. GWAS enabled the availability of genetic variants and genes mediating complex disease, and risk estimation models are now being built using genetic data as part of their parameters [86, 211]. Pharmacogenomics risk models are currently explored in the setting of neurodegenerative diseases [212]. Candidate-gene-set-based risk estimation using genetic variants in pro-inflammatory genes and PCa genes have also been suggested [213], but some of these recommendations were not replicated when tested using whole-genome or exome-wide approaches. Nevertheless, combining large patient population and mapping subpopulation specific risk would help to delineate pharmacogenomic variants and genetic predisposition to chemotherapy responses.

Fig. (8). Drug repositioning of cancer drugs mapped across different disease groups across International Classification of Disease – v9 coding system. Diseases categories are labeled using green font, name of medications are labeled in black.
**DISCUSSION**

Delivering the right drug for the right patient in the right dose at the right time via the right route is a critical step in implementing precision therapy in a clinical setting. Recommending cancer treatments by leveraging variations in pharmacogenes (genes associated with metabolism of drugs) and mutation landscape of tumor would help to develop precision therapy. NEPC is an aggressive cancer phenotype that evolves via complex and largely unknown mechanism and patient communities can benefit from such approaches. Typically, NEPCs do not express AR, and subsequently prostate-specific antigen (PSA) used for PCA screening. Thus, early diagnosis of NEPC is challenging, under-reported, and, by the time diagnosis is made, therapeutic interventions and treatment stratification are limited. Furthermore, since PSA is also used to monitor biochemical recurrence during and after treatment, development of treatment-induced NEPC is under appreciated. Recent data suggests that upwards of 25% of patients treated with new generation antiandrogen signaling therapies targeting signaling modules progress towards NEPC [205, 214]. Thus, there is an unmet and urgent clinical need to not only develop better biomarkers for detecting NEPC early, but also for treatment strategies to overcome NEPC development. With an increasing number of diagnoses, limited treatment options, and high mortality rates, NEPC patient populations need better treatment modalities faster than the typical drug development processes deliver them. We discussed a variety to approaches to tackle NEPC, but other integrated approaches can also be used to understand and treat the NEPC. For example, designing RNA interference based single molecule or gene-set based inhibition could help to find key players of NEPC. Drug screening using viability assays and chemical genomics of NEPC cell lines would then help to find primary candidates for NEPC. Developing better cell lines, patient-derived xenograft (PDX) models and other mouse models, and leveraging high-throughput sequencing could help to find new targets, processes and pathways of NEPC.

**CONCLUSION**

With the advent of precision phenotyping and personalized risk assessment, we see the rapid evolution of cancers and cancer subtypes averse to standard treatment methods. Better therapies that consider the dynamic characteristics of tumor evolution and tumor volatility to evolve, and emerge as metastatic cancer phenotypes needs to be addressed. Studies are needed on systems scale using deep profiling techniques to understand the disease trajectory of the evolution of NEPC from PCa. Computational approaches including systematic drug repurposing, drug-modeling, metabolic modeling, developing protein or peptide therapies, and the use of nanoparticles could play a significant role in discovering and developing effective therapies to target NEPC. In addition to the curative strategies, developing predictive models could aid to understand the individual risk of patients to develop chemotherapy resistance and NEPC. An integrated pharmacological risk assessment that uses pharmacogenomic profiling with tumor profiling would also be helpful to predict response to therapies. Developing personalized risk estimates using genetics, family history, diet, environmental exposure and behavioral end points would help to develop precision risk models. To conclude, developing precision risk models that use pharmacogenomic profiling, lifestyle data (including exercise, diet, and environmental exposure) coupled with deep molecular profiling using genomics, transcriptomics, metabolomics and proteomics will elaborate a more comprehensive, systems-wide understanding of NEPC and ultimately enable predictive and therapeutic strategies.

**AUTHORS’ CONTRIBUTION**

KKY and KS wrote the first draft manuscript. BR, SSY, LL and AK provided critical inputs. KS compiled the data and performed the empirical analyses. KKY and SSY performed in vitro assays and model of NEPC differentiation. AT and JTD contributed to the overall planning of the analyses and the manuscript. All authors read and approved the final manuscript.

**CONFLICT AND FINANCIAL INTEREST**

KKY, KS, SSY: None declared;

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**REFERENCES**


Spiotto MT, Chung TD. STAT3 mediates IL-6-induced neuroendocrine transdifferentiation in prostate cancer cells. Proc Natl Acad Sci USA 1998; 95(7): 3644-49.


Fedoroff HJ, Gostin LO. Evolving from reductionism to holism: is there a future for systems medicine? JAMA 2009; 302(9): 944-6.


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[81] Prostate Adenocarcinoma [http://cancergenome.nih.gov/ cancersslected/prostatecarcinoma]"


