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INVITED REVIEW

Prostate Cancer

Molecular signaling involving intrinsically disordered proteins in prostate cancer

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Investigations on cellular protein interaction networks (PINs) reveal that proteins that constitute hubs in a PIN are notably enriched in Intrinsically Disordered Proteins (IDPs) compared to proteins that constitute edges, highlighting the role of IDPs in signaling pathways. Most IDPs rapidly undergo disorder-to-order transitions upon binding to their biological targets to perform their function. Conformational dynamics enables IDPs to be versatile and to interact with a broad range of interactors under normal physiological conditions where their expression is tightly modulated. IDPs are involved in many cellular processes such as cellular signaling, transcriptional regulation, and splicing; thus, their high-specificity/low-affinity interactions play crucial roles in many human diseases including cancer. Prostate cancer (PCa) is one of the leading causes of cancer-related mortality in men worldwide. Therefore, identifying molecular mechanisms of the oncogenic signaling pathways that are involved in prostate carcinogenesis is crucial. In this review, we focus on the aspects of cellular pathways leading to PCa in which IDPs exert a primary role.
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INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed cancer and the sixth leading cause of cancer deaths in males.[1,2] In recent years, several targets have been identified and current treatment methods for PCa include radical prostatectomy or radiation, androgen deprivation therapy (ADT), and chemotherapy. However, most patients treated with ADT develop castrate-resistant PCa (CRPC).[3] Hence, there is a great interest in unveiling the molecular events that are crucial for the development of the disease and in introducing new agents for the treatment of its advanced stages. Targeting the androgen receptor (AR) signaling pathway is the current standard treatment for hormone-naive CRPC. After 2–3 years, however, AR signaling is reactivated leading to castrate-resistant PCa.[4] Abiraterone acetate and enzalutamide represent the next-generation of antiandrogen agents and are routinely used in the clinical treatment compared to flutamide or cyproterone acetate which are on a significant decline in the chemotherapy.[5–7] Cabazitaxel was approved for CRPC after docetaxel relapse.[8] Recently, radium-223 chloride (radium-223) was approved for the treatment of metastatic CRPC.[9] **Table 1** provides an overview of drugs currently approved for treatment of CRPC and their molecular targets as well as the overall survival (OS) and the progression-free survival (PFS) rates. These data underscore the dire need for new and effective therapeutics to treat and manage PCa.[10]

Recent investigations on protein interaction networks (PINs) revealed that proteins that constitute hubs in a PIN are more disordered compared to proteins that constitute edges (schematic representations of selected PINs are presented in **Figure 1**). These proteins are known as intrinsically disordered proteins (IDPs)[11–22] or hybrid proteins possessing both structured domains and disordered protein regions (IDPRs). Their structural plasticity and conformational adaptability to changes in their environment, binding promiscuity and unique capability to fold differently while interacting with different binding partners[23,24] define a wide set of functional advantages of IDPs/IDPRs over fully ordered proteins. These factors determine the abundant involvement of IDPs/IDPRs in various signaling, regulatory, and recognition processes. Furthermore, deregulation of disordered proteins leads to protein misfolding, misidentification and inaccurate signaling promoting numerous human diseases such as cancer, cardiovascular disease neurodegenerative diseases, and diabetes.[25–27] Several cellular mechanisms such as chromosomal translocations, aberrant splicing, altered expression, posttranslational modifications, aberrant proteolytic degradation, and defective trafficking are some of the factors that induce pathogenic transformations of IDPs.[28] The misfolding of many proteins is often accompanied with protein aggregation causing several human diseases that originate from the deposition of protein aggregates formed from specific proteins or protein fragments, which accumulate in a variety of organs and tissues.[29–37] In contrast, several diseases are caused by misfolding and, therefore, dysfunctional proteins.[17,38,39]

Several well-known cancer-related proteins with experimentally confirmed IDPRs include p53,[40] BRCA1,[41] HPV protein,[42] PTEN,[43] and an overwhelming majority of the Cancer/Testis Antigens (CTAs).[44] Bioinformatic approaches allow investigators to predict the presence of IDRs in natural proteins by assembling specific datasets of proteins associated with a given disease and by computationally analyzing these datasets using a number of disorder predictors.[38] This approach has revealed that the majority of proteins involved in eukaryotic signal transduction are IDRs, and further, 79% of cancer-associated and

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Table 1: Drugs currently approved for metastatic CRPC

Drug	*Molecular target*	*OS (months)*	*PFS (months)*
Docetaxel	Tubulin	18.9 *versus* 16.5	Not assessed
Abiraterone	Androgen receptor	34.7 *versus* 30.3	16.5 *versus* 8.3
Enzalutamide	Androgen receptor	32.4 *versus* 30.02	Not reached *versus* 3.9
Sipoleucel T	Immune system	25.9 *versus* 21.4	11.7 *versus* 10
Enzalutamide postdocetaxel	Androgen receptor	18.4 *versus* 13.6	8.3 *versus* 2.9
Abiraterone postdocetaxel	Androgen receptor	15.8 *versus* 11.2	5.6 *versus* 3.6
Radium-223	Symptomatic bone metastases	14.9 *versus* 11.3	3.6 *versus* 3.4
Cabazitaxel	Tubulins	15.1 *versus* 12.7	2.8 *versus* 1.4

OS: overall survival; PFS: progression free survival; CRPC: castrate-resistant prostate cancer

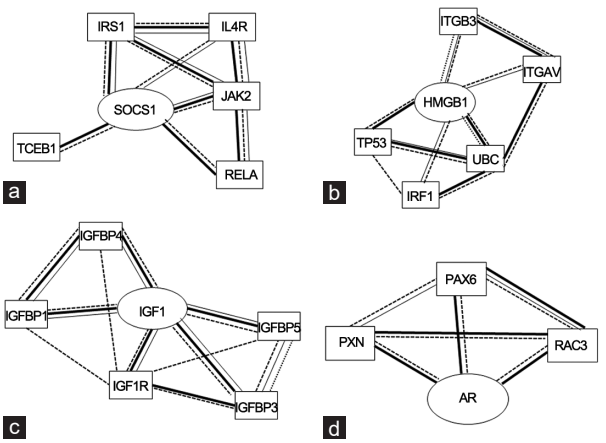

Figure 1: Schematic representation of PIN (protein interaction networks) of several proteins investigated in this review: (**a**) SOCS1, IRS1: insulin receptor substrate 1, IL4R: interleukin 4 receptor, JAK2Janus kinase 2, RELAv-rel: reticuloendotheliosis viral oncogene homolog A (avian), TCEB1: transcription elongation factor B (SIII), (**b**) IRF1: interferon regulatory factor 1, ITGB3: integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61), ITGAV: integrin, alpha V, TP53: tumor protein p53, UBC: ubiquitin C, (**c**) IGFBP1: insulin-like growth factor binding protein 1, IGFBP4: insulin-like growth factor binding protein 4, IGFBP5: insulin-like growth factor binding protein 5, IGF1R: insulin-like growth factor 1 receptor, IGFBP3: insulin-like growth factor binding protein 3, (**d**) PAK6p21 protein, (Cdc42/Rac)-activated kinase 6, PXN: paxillin, RAC3: ras-related C3 botulinum toxin substrate 3.

66% of cell-signaling proteins contain predicted regions of disorder of thirty residues or longer.[19,23] Similarly as many as 40%–50% of all eukaryotic genes are predicted to encode proteins containing lengthy disordered segments (>forty residues).[45,46] These proteins are usually involved in molecular recognition and assembly, protein modification (e.g., phosphorylation, acetylation, methylation) and entropic chain activities (e.g., linkers, springs, and spacers). In contrast to globular proteins, IDPs usually are constituted by only single continuous segments designated molecular recognition elements (MoRE/MoRF) or linear motifs, whereas the binding sites of ordered proteins are more segmented.[25] These motifs are short, conserved within larger protein segments that function as sites of regulation, and many are posttranslationally modified.

Chromosomal translocation causes several forms of cancer, such as acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), primary myelofibrosis (PMF), anaplastic large cell lymphoma, non-Hodgkin's lymphoma, Ewing's sarcoma (EWS), colorectal cancer (CRC), nonsmall cell lung cancer, lung adenocarcinoma, and sporadic and radiation-associated papillary thyroid carcinomas. Computational analysis of the 406 translocation-related human proteins revealed that these cancer-related proteins are enriched in disordered regions, with the translocation breakpoints mostly located outside the functional domains.[47] In this scenario, although the flexibility of Alternative Splicing (AS) represents an evolutionary advantage for higher eukaryotes, it also represents a risk. Indeed, defective AS regulation correlates with the onset and progression of human cancers, and many cancer-associated genes are regulated through AS suggesting a significant role of this posttranscriptional regulatory mechanism in the production of oncogenes and tumor suppressors.[48–50] Most proteins resulting from aberrant splicing are enriched in intrinsic disorder, such as those involved in apoptosis[51] and in spliceosome assembly.[7,52,53]

Some of the crucial proteins affected by AS in PCa (e.g., AR, zinc finger transcription factor, Kruppel-like-factor 6, Bcl-x, and cyclin D1)[54] are known to contain IDPRs. A case of extensive AS of the *TMPRSS2-ERG* gene fusion represents an important illustration of the combined effects of chromosomal translocation and AS in cancer progression.[54,55] ETS-related gene (ERG), which is a member of the erythroblast transformation-specific (ETS) transcription factor family, is typically expressed at very low levels in benign prostate epithelial cells but, when fused with the androgen-responsive transmembrane protease serine 2 (TMPRSS2), generates a PCa oncogene. Furthermore, this fusion-derived gene undergoes AS and generates multiple mRNA variants encoding both full-length ERG proteins and isoforms lacking the ETS domain. Notably, an increase in the abundance of transcripts encoding full-length ERG was shown to correlate with less favorable outcomes in PCa patients.[55] Thus, it follows that a plethora of cellular events are responsible for initiation and progression of PCa, but most of them remain poorly understood. Here, we focus on some of the important molecular mechanisms associated with oncogenic signaling pathways that are involved in prostate carcinogenesis namely, inflammation, insulin-like growth factor axis, and androgen receptor signaling pathway.

INFLAMMATION

SOCS proteins in JAK-STAT cellular pathway

There are several types of cells in the prostate, but nearly all PCas start in the gland cells. This kind of cancer is known as adenocarcinoma.[45,46] Currently, other than grading by Gleason Score, there are no effective biomarkers to discern patients with indolent disease from those with aggressive disease which needs to be treated immediately. Investigations by several labs indicate that chronic inflammation is a known contributor to several forms of human cancer, with an estimated 20% of adult cancers attributable to chronic inflammatory conditions caused by infectious agents, chronic noninfectious inflammatory diseases and or other environmental factors. Thus, chronic inflammation is now regarded as an 'enabling characteristic' of human cancer.[56,57] In PCa, chronic inflammation has been postulated to be a trigger for epithelial to mesenchymal transition (EMT), a process that leads to increased CRPC and drug resistant disease. In fact, infiltration of the tumor tissue with M2 macrophages has been shown to induce an EMT, just as these wound-healing macrophages do in normal cells after a cut. This process is visible on histological examination by white blood cells, increased Gleason Score, and certain markers; however easily assayable early detection of EMT in PCa has remained elusive.

Since the Janus kinases/signal transducer and activator of transcription factors (JAK-STAT) pathway play a key role in the

inflammatory reaction in cancer,[58] particular attention is focused on the role of the negative regulators and suppressor of cytokine signaling proteins (SOCSs) of STATs. The SOCS family comprises eight members, SOCS-1 to -7 and CIS. SOCS family members share the central Src homology 2 domain and SOCS box in the carboxy-terminal which play a crucial role in proteasomal degradation of binding partners[59–62] (**Figure 2a**). The N-terminal domains of SOCS proteins vary in length and amino acid sequence, and only SOCS-1 and SOCS-3 possess a kinase inhibitory region (KIR, 17 residues) immediately upstream of the central SH2 domain.[63] SOCS proteins attenuate cytokine signal transduction by binding through their SH2 domains to phosphorylated tyrosine residues on signaling intermediates, such as receptor subunits and JAKs. Thus, the binding of SOCSs to JAK kinases blocks further signaling in a negative feedback loop.[64] The structures of SOCS-3 in complex with a phosphotyrosine-containing peptide from the interleukin-6 (IL-6) receptor related to the signaling subunit gp130,[65] with JAK2[66] and those of ternary complexes of SOCS-2, -3 and -4 associated with elongin B and elongin C[67,68] have been reported. However, the structure and function of the N-terminal domains of the SOCS proteins remain poorly characterized. However, they have been suggested to mediate the ubiquitination of substrate proteins bound to their N-terminal domains and peptide, and peptidomimetics covering these unstructured regions revealed potential anti-inflammatory properties.[69–72] Furthermore, the N-terminal domains of SOCS-4 and -5 play an important role in mediating interactions with the epidermal growth factor receptor and interleukin-4 receptor. N-terminal domains have been predicted and experimentally demonstrated as internally disordered regions (**Figure 2**).[73] In **Figure 2a**, a schematic representation of SOCSs structure and the profile of disorder prediction for the whole human sequences of SOCS-1 and -3 (using PONDR-FIT[74]) (**Figure 2b** and **2c**) are reported. Our results appear similar to those related to other disorder predictors (such as VSL2[45]). The altered expression of SOCS-1 and -3 in PCa[75,76] was recently confirmed by analyzing the expression and localization of STAT and SOCS-1 proteins using immunohistochemistry on 150 Formalin-fixed and paraffin-embedded human prostate tissues of different grades; fundamental differences in major oncogenic signaling cascades between benign and malignant form of prostate tissue were observed. A critical association between altered expression of STAT-3 and -5 with SOCS-1 suggested for the latter a potential role as a negative regulator independent of JAK-STAT pathway in tumorigenic transformation of prostate tissue.[77] The growth-inhibitory effects of SOCS-1 in human PCa cell lines were also demonstrated by the use of the SOCS-1 mimetic peptide Tkip that acted as a negative growth regulator of both DU-145 and LNCaP cells.[69] These studies unveiled that the SOCS-1 inhibitory effect is mediated through negative regulation of STAT3 phosphorylation and that its down-regulation increased expression of cyclins D1, cyclins E, cdk 2 and cdk 4, which drive cell cycle progression to S phase. Differential regulation of SOCS-1 in PCa cell lines may be explained by either the presence of AR mutations or insufficient expression of a coactivator, which is critical for SOCS-1 expression. At present, it could be hypothesized that SOCS-1 down-regulation affects growth regulation by androgens in PCa.[78] Further SOCS-1, if expressed in PCa cells, has a growth-regulatory role in this malignancy. Although the presence of both SOCS-1 mRNA and protein was detected in all tested cell lines, expression levels decreased in samples taken from patients undergoing hormonal therapy but increased in specimens from patients who failed this therapy. In LNCaP-interleukin-6 PCa cells, SOCS-1 was upregulated by interleukin-6 and in PC3-AR cells by androgens; such upregulation was also found to impair cell proliferation. In contrast, down-regulation of SOCS-1 expression caused a potent growth stimulation of PC3, DU-145, and LNCaP-IL-6 cells that were associated with the increased expression levels of cyclins D1 and E as well as cyclin-dependent kinases 2 and 4. Other studies demonstrated that the down-regulation of SOCS-3 causes cell death of PCa cells through activation of the extrinsic and intrinsic apoptosis pathways.[79] The underlying mechanism is that SOCS-3 antagonizes the proliferative and migratory ability of PCa cells by inhibition of p44/p42 MAPK signaling.[80] In addition, SOCS-3 inhibits the signal transducer and activator closely related to PCa cell proliferation and invasiveness, such as STAT3.[81]

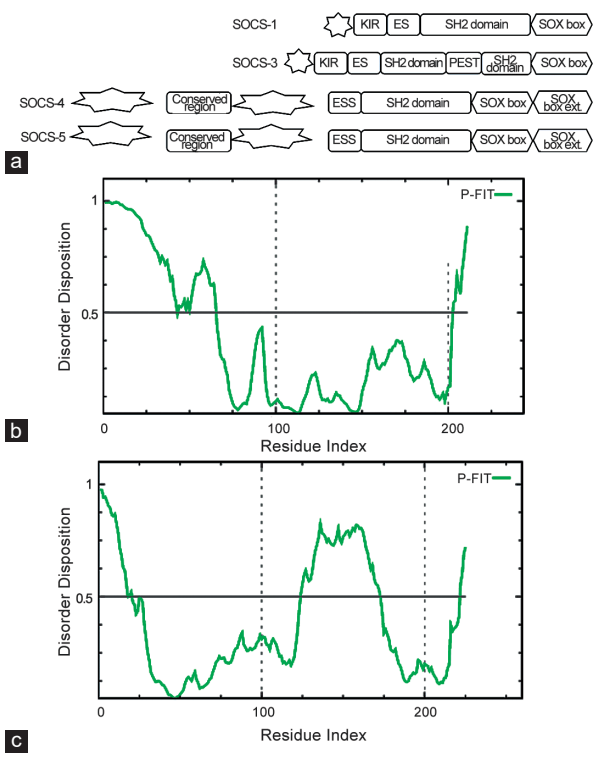

Figure 2: (**a**) Schematic representation of SOCS proteins modular structures, IDRs are indicated as stars shape. Prediction of disorder tendency of (**b**) SOCS-1, (**c**) SOCS-3 sequences with PONDR-FIT.

In addition, SOCS-3 inhibits PCa cell growth and Liver X receptors (LXRs) agonists may inhibit the carcinogenesis of PCa via the SOCS-3. In cells treated with control-siRNA, indeed the inhibitor GW3965 enhanced SOCS-3 expression and inhibited the phosphorylation of STAT3, NF-κB and AP1 expression, accompanied by dramatically reduced cellular proliferation rate, immigration and invasion of cultured cells.[82,83]

Interestingly, when the interaction between the tumor-selective apoptosis inducer tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and SOCS-3 was investigated, it was observed that SOCS-3 appeared as one of the proteins which influence the ability of TRAIL and resveratrol to cause programmed cell death in PCa.[84]

HMG1: a proinflammatory cytokine

HMGB1 is secreted by immune cells (like macrophages, monocytes and dendritic cells) through leaderless secretory pathway.[85] HMGB1 is also a DNA binding protein involved in its replication and repair process[86,87] but is secreted by activated macrophages and monocytes

as a cytokine mediator of inflammation.[88] HMGB1-RAGE axis plays a major role in inflammation induced by carcinogenesis:[89,90] in ovarian cancer and PCa, different isoforms of the human HMGB1, encoded by the *HMGB1* gene, have been reported[91] and other genes (*HMGB-2*, *-3* and *-4*) encoding similar, although less studied HMGB proteins, are present in the human genome.

HMGB1 is highly expressed in PCa cells[92] and targeting HMGB1 disrupts tumor progression by inhibiting activation of T-cells and reducing infiltration of macrophages which are considered to be key inflammatory cells in promoting variety of cancers including PCa.[93] Interestingly, androgen deprivation caused HMG1's secretion in prostatic stromal cells supporting the notion that deprivation therapy may upregulate the expression of HMGB1 leading to either hormone resistance or metastatic disease.[94]

HMGB1 is the most studied member among the human HMGB protein family. It has many different functions that depend on its redox state and posttranslational modifications, including acetylation, which determine its cellular or extracellular localization. HMGB1 is polyacetylated near its nuclear-localization sequences (NLSs) and this modification blocks the interaction with nuclear importer proteins.[95] In addition, HMGB proteins have a common modular structure, reported in **Figure 3a** with two positively charged DNA binding domains, HMG A-box and HMG B-box, folded in the characteristic L-shaped architecture. Each domain is formed by three alpha helix-stretches.

In HMGB1, the HMG A-box includes amino acids 1–79, and the HMG B-box spanning amino acids 89–163. The acidic C-terminal domain (186–215) is negatively charged with an extended and flexible sequence (as bioinformatics analysis in **Figure 3b**), which constitutes the intrinsically disordered region[96] and can interact with residues within and between the two HMG boxes[97] although it has the highest affinity for the HMG B-box.[98] Different functions of HMGB1 are associated with redox changes making it a master redox sensor. This function depends on three cysteine residues (at 23, 45 and 106 positions): Cys^{23} and Cys^{45} easily form an intramolecular disulfide bridge, while Cys106 remains reduced (the semi-oxidized HMGB1 form). The formation of this disulfide bond is thermodynamically favored; thus, a significant fraction of HMGB1 is in the semi-oxidized form within cells.[99] The proximity of the Cys residues to residues required for DNA binding[100] highlights the importance of redox-regulated conformational changes in HMGB1, which may modulate their affinity for DNA. Thus, redox changes affect the interaction with other proteins and modify their biological functions. The structural analysis of HMGB1 A-box reveals that Cys23 and Cys45 are located at the center of helix I and helix II, respectively, opposing each other and at a distance that allows the formation of a disulfide bond under appropriate oxidative conditions. Two NLSs, rich in lysine residues and spanning 28–44 and 179–185 regions, respectively, can be specified.[101] Although Cys106 is not located within the NLS, thiol may participate in nuclear transport through nuclear pore complex binding, ubiquitination, or transporter interaction[102] and is important to preserve the nuclear functions of these proteins. Taken together, these data suggest that the intrinsically disordered HMG protein may represent novel targets in PCa.

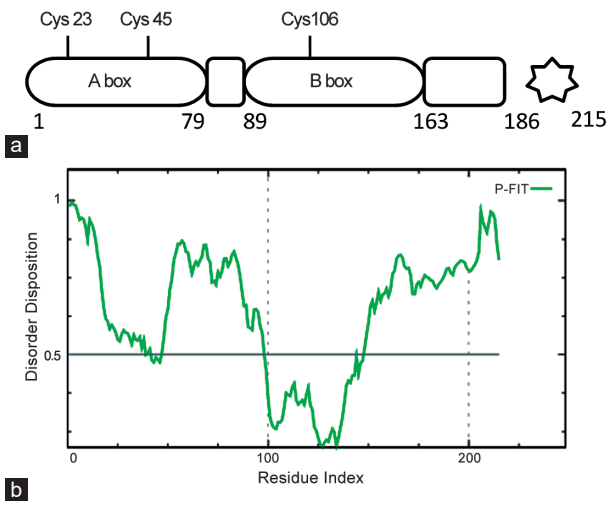

Figure 3: (**a**) Schematic representation of HMGB1 modular structure, IDRs are represented by a star shape, (**b**) Prediction of disorder tendency of HMG1 sequence with PONDR-FIT.

IGF AXIS SIGNALING

Interest in insulin-like growth factors (IGFs) and their effect on carcinogenesis has increased recently because high serum concentrations of IGF1 are associated with an increased risk of breast, prostate, colorectal and lung cancers. Physiologically, IGF1 is not only the major mediator of the effects of the growth hormone strongly influencing cell proliferation and differentiation, but it is also a potent inhibitor of apoptosis.[103]

IGFs are proteins with high sequence similarity to insulin and are part of a complex system that cells use to communicate with their physiologic environment. This system, "IGF axis" consists of two cell-surface receptors (IGF1R and IGF2R), two ligands IGF-I and IGF-2, a family of six high-affinity IGF-binding proteins (IGFBP-1 to IGFBP-6), as well as associated IGFBP degrading enzymes, referred to collectively as proteases.[104] The IGF axis is one of the most investigated pathways in cancer and mediates critical physiological processes, including cellular growth, cell differentiation, apoptosis, development, as well as glucose and lipid metabolism.[105] Moreover, the IGF axis plays a fundamental role in regulating embryonic growth and IGFs also exert growth stimulating and pro-survival effects on tumor cells where they have been shown to mediate carcinogenesis, angiogenesis, malignant cell proliferation and metastatic growth of a variety of tumors.[106] To date, more than 100 agents have been developed to target the receptors of the IGF axis and many are currently being evaluated in preclinical and clinical studies. These investigations have demonstrated that the IGF axis modulates the development and progression of PCa. Indeed, IGF1 signaling is elevated in PCa compared to benign prostate tissue and exerts a role in tumor progression.[107] Data obtained from mouse cells with a disrupted IGF1R gene suggest that this receptor is a prerequisite for cells to undergo oncogene-induced transformation. Consistently, inhibition of IGF1R shows therapeutic efficiency in a number of preclinical models of hormone-dependent tumors, including those of the prostate and breast.[108] Epidemiological data suggest that high circulating IGF1 levels are associated with a moderately increased risk for PCa development. Pharmacological inhibition strategies include growth factor entrapping monoclonal antibodies, employing kinase defective mutant receptors, antisense oligonucleotides, growth factor sequestrating IGFBPs, soluble forms of the receptor, receptor neutralizing antibodies and small-molecule tyrosine kinase inhibitors of IGF1R and INSR.[109–111]

The solution structure of IGF1 was investigated with a combination of nuclear magnetic resonance and restrained molecular dynamics methods. The results show that it is quite similar to that of insulin, but minor differences exist. In detail resonance assignments were hampered by the lack of spectral dispersion and broad line widths, arising from

conformational averaging and the tendency of IGF-I to aggregate.[112,113] The regions homologous to insulin are well-defined, well conserved and confined in the first 27 amino acids of the sequence as reported in **Figure 4a**. Clearly, the N-terminal domain is the most important to its function, while the remainder of the molecule exhibits greater disorder and has been demonstrated to contain the sites for interaction with a number of physiologically important proteins.[114,115]

ANDROGEN RECEPTOR SIGNALING

AR is a member of the nuclear receptor (NR) superfamily of ligand regulated transcription factors that plays an integral role in primary and secondary male sexual development.[116] As a key regulator for the growth, terminal differentiation and function of the prostate gland, AR has important functions in regulation of proliferation, cell cycle, apoptosis, angiogenesis and differentiation in PCa.[117–119] Identified as a primary target for the treatment of PCa, many therapeutic strategies were developed to attenuate AR signaling. While frontline androgen-deprivation therapies targeting either the production or action of androgens usually yield initially favorable responses in PCa patients, a significant number acquire treatment resistance.[120] The signaling is restored in castrate-resistant prostate cancer (CRPC) due to many aberrant mechanisms such as AR mutations, amplification, or expression of constitutively active splice-variants. Structural analyses of the AR have identified key surfaces involved in protein–protein interaction with co-regulators that was used to design and develop promising AR-coactivator binding inhibitors for the treatment of PCa. The currently used AR antagonists, such as flutamide, bicalutamide, nilutamide, and enzalutamide (MDV3100) (**Table 1**), act by binding to the androgen binding site (ABS) of the AR, resulting in conformational changes that prevent its activation. In the absence of ligands, the AR predominantly resides in the cytoplasm where it is associated with heat shock proteins in a transcriptionally inactive form until it is activated by testosterone or the more potent metabolite, 5a-dihydrotestosterone (DHT).[121] Upon ligand binding, the AR undergoes a substantial conformational change leading to dissociation from repressor proteins, dimerization, translocation to the nucleus and association to androgen-responsive elements (ARE) in the regulatory regions of target genes.[122] Through this regulatory mechanism, the AR regulates the expression of more than a thousand genes including PSA, which is the most well-known biomarker for PCa.

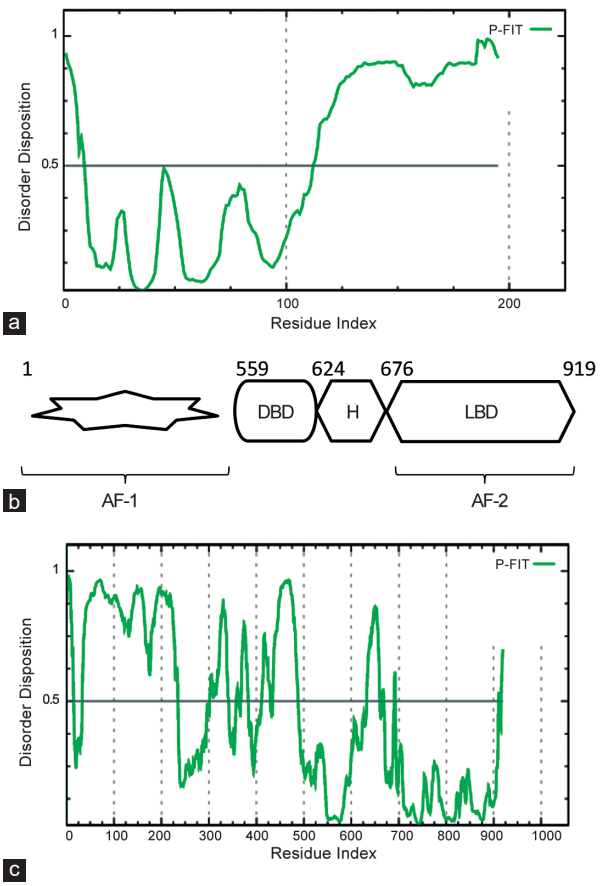

Figure 4: (**a**) Prediction of disorder tendency of IGF1 sequence with PONDR-FIT, (**b**) Modular structure of the AR, IDRs are represented by a star shape (**c**) Prediction of disorder tendency of AR sequence with PONDR-FIT.

Similar to other nuclear receptors (NRs), the AR displays a modular structure composed of an N-terminal domain (NTD) bearing the activation function (AF-1), a DNA-binding domain (DBD), a connecting hinge region containing a nuclear localization signal (NLS) and a C-terminal ligand-binding domain (LBD) (**Figure 4b**).[123] The LBD is constituted by 12 anti-parallel α-helices with the ABS buried inside which undergoes conformational rearrangement upon agonist binding. The helix 12 (H12) is the most flexible part of the LBD and its repositioning after androgen binding completes the AF-2 binding surface by creating a hydrophobic groove that allows the docking of leucine-rich, LXXLL motif-containing co-regulatory proteins.[124] In addition, the intrinsic dipole moment of the co-activator α-helix is matched on the AF-2 by a negatively charged residue E897, on H12, at the N-terminus and a positively charged K720, on H3, at the C-terminus, which form a charge clamp.[125] The LXXLL motif, named the NR box, is present in several coactivators such as proteins of the p160 family.[126] Mainly formed by residues from helices 3, 4, 5, and 12, the AF-2 surface on the AR-LBD is unique among NRs in preferring to interact with the more bulky hydrophobic motifs F/WXXLF respect to the LXXLL. These interdomain interactions play an important role in selective AR-dependent gene regulation but are disrupted by DNA binding, which in turn would expose the NTD and AF-2 surfaces for interactions with co-regulators.[127] Also known as the coactivator binding pocket, the AF-2 is the major protein–protein interaction (PPI) surface used by NRs for coactivator recruitment. While AF-2 is a privileged site to develop inhibitors of AR-protein-protein interactions (PPIs), other sites were also recently exploited.[128]

The AR-NTD plays an essential role in ligand-dependent AR transcriptional activity but, in addition, has a ligand-independent transactivation function.[129] Indeed, C-terminal truncated ARs lacking the LBD retain a constitutive activity and such splice variants can be observed in CRPC.[130,131] The AF-1 region located in the NTD contributes to these functions by interacting with other proteins such as components of transcription factors (TF) IIF and IIH and co-activators SRC1-3 and CREB-binding protein (CBP).[131] The intact NTD domain (AR1–558) resulted in being able to inhibit both ligand-dependent and independent transcriptional activities by sequestering co-regulatory proteins.[132] The NTD is the least conserved domain across steroid receptors with <15% homology and very few disease-associated mutations of the AR have been reported in this domain.[133] Thus, the AR- NTD represents a very attractive target to develop inhibitors that could block ligand-dependent and ligand-independent AR transactivation and hence, be active in hormone-sensitive and castrate-resistant PCas. Nevertheless, the design of inhibitors targeting the AF-1 site is partially hampered by the lack of structural information for the NTD, which is highly flexible and

intrinsically disordered (**Figure 4c**). However, little is known about the functional dynamics of the NTD in PCa cells due to a high degree of intrinsic disorder in this domain.[134] Previous studies reported on helices in AR transcriptional activation, a ^{178}LKDIL^{182} motif resident in the AF1a region of the TAU1 domain[135,136] and a ^{435}WHTLF^{439} motif in the TAU5 domain.[137] Following a combinatorial approach that is the most applied in these cases[71,138,139] to target the AR-NTD, a library of marine sponge extracts were screened in a transactivation assay.[140] Several short chlorinated peptides from the sponge *Dysidea* sp. named Sintokamides were identified and no cytotoxicity was observed in a cell viability assay. Sintokamide A 15 was found to reduce PSA expression and block AR-NTD transactivation at 5 mg ml^{-1} in luciferase reporter gene assays and to inhibit proliferation of AR-positive LNCaP cells but not AR-negative PC3 cells. Another screen was more recently performed on extracts of the marine sponge *Niphates digitalis* leading to the discovery of niphatenones, a group of glycerol-ether lipids, as AR-NTD inhibitors at different disease stages.[141]

Important advances in the structure-function relationships of the AR and structural interactomic studies of AR splice variants in PCa evolution have provided a detailed knowledge of the AR axis in PCa. The limited treatment options for CRPC patients and the increasing appreciation of the role of AR in advanced PCa prompt the development of new approaches to target AR signaling. Different strategies to destabilize the AR, prevent its nuclear translocation, inhibit its binding to DNA or block co-activator recruitment are being explored. The latter, mediated by PPIs, offers the opportunity to develop selective noncompetitive AR antagonists that would overcome resistance to traditional anti-androgens and remain effective in advanced PCa. PPI inhibitors have been developed using a wide variety of approaches including structure-based drug design, screening of natural compound libraries, ligand-based peptidomimetics and different combinations of virtual library screening and experimental evaluation. While many compounds described need more comprehensive SAR studies and pharmacological optimization to progress into clinical evaluation, some including EPI-001 and oligobenzamides have already shown very encouraging results *in vivo*.[142]

In this scenario, an important study focused on the modulation of AR functions by nucleophosmin1 (NPM1/B23), a member of the histone chaperone family, that in turn contains an IDR. Further NPM1 resulted overexpressed in PCa.[143] NPM1 is an abundant multifunctional protein which is present in high quantities in the granular region of nucleoli.[144] It is capable of shuttling between the nucleus and cytoplasm and is involved in many cellular functions such as the regulation of ribosome biogenesis, chromatin remodeling, DNA replication, recombination, transcription repair, and the control of centrosome duplication.[145] Notably, NPM1 has been identified as the most frequently mutated gene in acute myeloid leukemia (AML) patients, accounting for approximately 30% of cases.[146] Besides its primary role as a therapeutic target in AML drug discovery programs, it represents an important model for multidomain proteins.[25,39,147] AR and NPM1 interact *in vitro* and *in vivo*, and NPM1 is critical for androgen-dependent transcriptional activation in LNCaP cells as an anti-NPM, siRNA downregulates transcription of a transfected ARE-containing reporter promoter as well as expression of the endogenous androgen-responsive PSA gene. NPM1 is highly co-expressed with the AR in secretory epithelial cells of localized PCas and strongly binds to the DNA-binding domain (DBD)/hinge domain of AR *in vitro* and facilitates AR binding to its consensus ARE suggesting that other proteins are probably involved in this AR–NPM1 interaction. In addition, it has been shown that AR binds to full-length NPM1 as well as to both the N-terminal domain and to the CTD-containing IDRs.[147] Deletion of these two regions abolished the interaction of NPM with AR indicating that the acidic/nuclear localization signal (Ac/NLS) domain of NPM1 is not sufficient for binding to AR. Altogether, these data indicate that AR and NPM1 interact *in vitro* through multiple domains and that other proteins seem to modulate this interaction *in vivo* and thus, even if IDRs of NPM1 are unable to directly bind to AR they seem to be crucial for the binding. Thus, the NPM1–AR interaction is linked to the regulation of gene expression by androgens during prostate carcinogenesis. Immunohistochemistry studies demonstrated that a strong and extensive staining for NPM1 was found in neoplastic prostate tissues while it is present at lower levels in the basal and luminal epithelial cells. Interestingly, AR also expresses more in the same sections of adenocarcinomas.[148]

CONCLUSIONS

In PPI networks disordered binding regions provide specific but transient interactions that enable IDPs to play central roles in important signaling pathways.[19] IDPs are endowed with crucial abilities to interact with multiple protein partners without losing specificity or affinity. The main challenging goal in this field lies in elucidating the structural basis for promiscuous binding and the mechanisms that lead to specific responses to a particular cellular signal.[149] The occurrence of intrinsic disorder in cancer-associated proteins strongly suggests that disorder needs to be seriously evaluated in the drug discovery process toward the development of novel therapeutic compounds. Unfortunately, this area has remained largely unexplored primarily due to the lack of effective screening tools. Structural and interactomic studies have helped to investigate pathways that are involved in prostate carcinogenesis and to unveil signaling events that are important for tumor progression. In addition to the ones that have been analyzed, many other PPIs involving IDPs have been selected as potential targets. For example, recently the Cancer/Testis Antigen (CTA), Prostate-associated Gene 4 (PAGE4) that is upregulated in PCa,[150] and several other CTAs such as the MAGE proteins, represent novel therapeutic targets for CRPC for which there are currently no effective therapeutics.[44,151]

COMPETING FINANCIAL INTERESTS

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

DM and EN conceived the manuscript; DM, AR, AM, and SLM wrote the manuscript.

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