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The Role of ETS Transcriptional Regulation in Hormone Sensitive and Refractory Prostate Cancer

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Abstract: Most advanced prostate tumors are dependent upon hormonal regulation of the transcriptional activity of the androgen receptor (AR). Current ASCO recommendations for initial treatment of advanced disease target the hormonal mediated regulation of AR activation through androgen deprivation therapy. Despite early treatment efficacy, most prostate tumors progress and re-activate AR transcriptional regulation through alternative biological mechanisms that allow them to circumvent the requirement for androgen. It is the temporal and spatial recruitment of specific AR transcriptional complexes to the promoters of cancer associated target genes that promotes the tumorigenic phenotype in prostate cancer. Increasing published data associates the E Twenty Six (ETS) family of transcription factors with prostate cancer progression and with the transcriptional activity of the AR**.** Evidence suggests that ETS factors act in concert to both positively and negatively regulate the pathways that control progression to metastatic cancer in prostate tissues. Given the critical roles both ETS factors and the AR play in the development of prostate cancer, mechanistic insight into their transcriptional co-regulation during the hormone sensitive and hormone refractory phases of progression will be provided by determining the contribution of ETS, AR and ETS-AR mediated regulatory control. This review examines the current depth of understanding of the role of the ETS family of transcription factors as transcriptional elements that confer the carcinogenic response to aberrant hormonal activity during prostate cancer progression.

Keywords: ETS factors, transcription, prostate cancer, hormonal regulation, androgen receptor, androgen deprivation therapy, hormone refractory prostate cancer.

HORMONAL CANCER

In the western world prostate and breast are the most commonly diagnosed cancers in men and women respectively. Combined, these two cancers account for around a third of all cancer related incidence and for the deaths of three quarters of a million people each year (http://who.int/research/en/). Significantly, around 70% of breast and 80% of prostate tumors are dependent upon hormonal regulation [1, 2]. While breast and prostate cancer are the most common and intensely studied of the hormone related cancers, ovary, endometrial, testicular, thyroid, colon, lung and pancreatic cancer also share a hormonal mechanism of progression [1, 2]. The molecular function of hormones is to maintain cell homeostasis. They are powerful regulators of cell survival and act to maintain the balance between cell growth and cell death through the regulation of the many inter- and intra-signaling pathways that mediate cell metabolism. Hormones bind to specific steroid receptors to initiate a chain of intercellular events that culminate in changes in transcriptional expression. Hormonally controlled gene expression therefore regulates the biological processes involved in maintaining the homeostatic state. During carcinogenesis aberrant hormonal regulation alters the homeostatic balance by increasing the expression of growth promoting genes while decreasing the expression of growth inhibiting genes to maintain cell survival and promote tumor progression. Therapeutic targeting of hormonal regulation alters the production and/or biological activity of specific cancer-related hormones and provides the foundation for many of the current breast and prostate cancer therapies.

HORMONE SENSITIVE PROSTATE CANCER

Improved early detection based, to a large extent, on prostate-specific antigen (PSA) screening has led to the identification of thousands of men with localized prostate cancer that can be treated by prostatectomy and/or radiation treatment (Fig. **1**) [3]. However, up to 20% of newly diagnosed prostate cancers are advanced by the time they are detected and at least 30% of patients treated for localized disease still progress to an advanced stage [3]. Most advanced prostate tumors are dependent upon hormonal regulation for growth and survival and are termed hormone (androgen) sensitive (Fig. **1**) [2, 4].

In normal prostate the androgen receptor (AR) is activated by the binding of the hormones (androgens) testosterone and 5α-dihydrotestosterone (DHT) and subsequently functions as a transcription factor [4]. Upon hormonal activation AR homodimers bind to hormonal response elements (HRE's) to regulate gene expression through the recruitment of additional transcriptional factors (Fig. **2A**). The AR transcriptional complex in turn modulates the basal transcription machinery to maintain gene expression and control cell homeostasis (Fig. **2A**) [4]. In hormone sensitive prostate cancer (HSPC), the AR preferentially recruits cancer specific co-regulatory factors to the AR transcriptional

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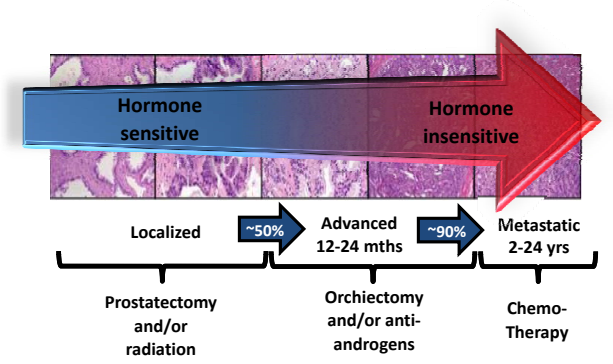

Fig. (1). The Three Phases of Prostate Cancer Treatment. Localized prostate cancer is treated by surgical removal of the prostate and/or radiation therapy. For advanced prostate cancer (androgen sensitive), AR activity is targeted through androgen deprivation therapy (orchiectomy and/or anti-androgens). Advanced prostate cancer which has become refractory to androgen deprivation (androgen insensitive) is treated by combinatory chemotherapy regimes. Even with treatment, survival rates are poor in patients with advanced refractory prostate cancer (2-24 months) [6,7].

complex resulting in aberrant transcriptional activation and increased expression of cancer promoting gene sets (Fig. **2B**). Current ASCO recommendations for initial treatment of advanced prostate cancer target AR activation through androgen deprivation therapy (ADT) (bilateral orchiectomy, medical castration, or both) [5]. ADT functions by lowering intracellular androgen levels to inhibit AR activation and reduce AR transcriptional activity [6]. However, over 80% of patients undergoing ADT no longer respond to treatment and progress to hormone refractory prostate cancer (HRPC) often within 12-18 months of diagnosis (Fig. **1**) [3].

HORMONE REFRACTORY PROSTATE CANCER

Patients diagnosed with HRPC have an extremely poor prognosis with progression-free survival rates as low as 2-24 months (Fig. **1**) [5, 7]. Combination chemotherapy with docetaxol and prednisone is the current first line standard of care for the treatment of HRPC and such taxane based regimes show a 20-24% decrease in the risk of death from HRPC but survival rates remain poor [7]. Due to these dire statistics there is an urgent need to gain a comprehensive understanding of the molecular underpinnings of HRPC to

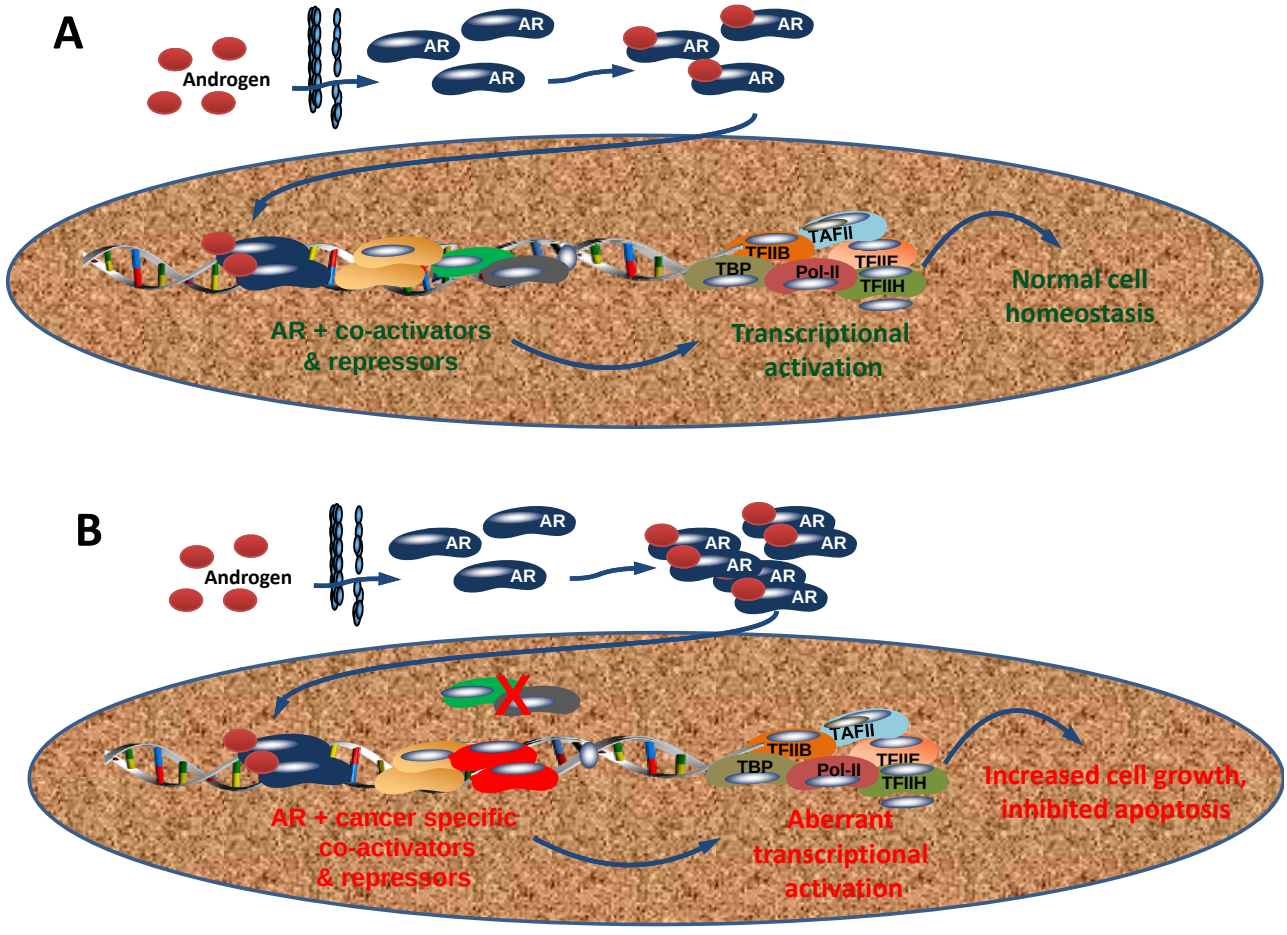

Fig. (2). AR Transcriptional Regulation in Normal and Transformed Prostate Cells. In normal prostate cells (**A**) the AR is activated by the binding of androgen and subsequently functions as a transcription factor. AR homodimers bind to androgen response elements to regulate gene expression through the co-recruitment of transcriptional co-factors at their consensus binding motifs which in turn regulates pre-initiation complex formation and transcriptional function to maintain cell homeostasis. During tumorigenesis (**B**) AR mediates the recruitment of a specific subset of co-regulatory factors which lead to the transcriptional activation and/or repression of cancer associated genes which promotes cell survival.

support, predictive, pre-emptive and personalized medicine for its treatment. Despite the insensitivity to androgen, the AR continues to play a critical role in promoting HRPC as reduced AR protein expression inhibits cell growth in both HSPC and HRPC molecular models [8]. A recent comparison of genome wide AR expression patterns and DNA binding elements identifies distinct molecular differences between the programs of AR recruitment and transcriptional regulation in hormone sensitive compared to hormone refractory prostate cancer [8]. AR promotes cell proliferation in hormone sensitive cells by promoting the G1/S transition of the cell cycle in an androgen dependent manner. In contrast, in hormone insensitive cells AR promotes cell proliferation through the direct recruitment of distinct AR co-regulatory factors to the regulatory elements of specific M-phase cell cycle genes in an androgen independent manner [8, 9]. No activation of M-phase genes was observed in the hormone sensitive cells in either the presence or absence of androgen and the results were confirmed *in vivo* using tissue microarray analysis of HSPC and HRPC tumor samples [8].

MECHANISMS OF THE HORMONE REFRACTORY PHENOTYPE

Upon the onset of hormone insensitivity, prostate cancer cells activate alternative biological mechanisms that allow them to circumvent the requirement for androgen and re-activate AR transcriptional regulation. Several mechanisms have been proposed to explain how prostate cancer circumvents androgen dependence and have recently been reviewed (Fig. **3**) [10]:

- Enhanced AR sensitivity lowers the threshold for androgen activation to compensate for low androgen levels;
- An increase in AR protein expression, as observed in xenograft tumors and in HRPC patients after ADT, is sufficient to augment the transition from androgen dependency to the androgen refractory phenotype and can cause AR antagonists to act as AR agonists;
- Prostate cancer stimulates the local production of androgen by the peripheral conversion of adrenal steroids;
- AR mutations reduce ligand-binding specificity to include other steroid androgens not affected by ADT;
- Alternative signaling cascades are activated upon reduced androgen levels that bypass hormonal control;
- ADT may select for cancer cells with intrinsic resistance to low androgen levels.

Significantly, the functional consequences of all of these mechanisms will ultimately be conferred by the temporal and spatial recruitment of specific AR transcriptional complexes to the promoters of cancer associated target genes (Fig. **3**). Sequence specific transcription factors such as AR, play a crucial role in transcriptional regulation by mediating complex formation at their consensus binding motifs (HRE's), which drives pre-initiation complex formation and transcriptional activation. In turn the correct recruitment of AR at the promoters of target genes is dependent upon spatial and temporal interactions with specific co-regulatory factors.

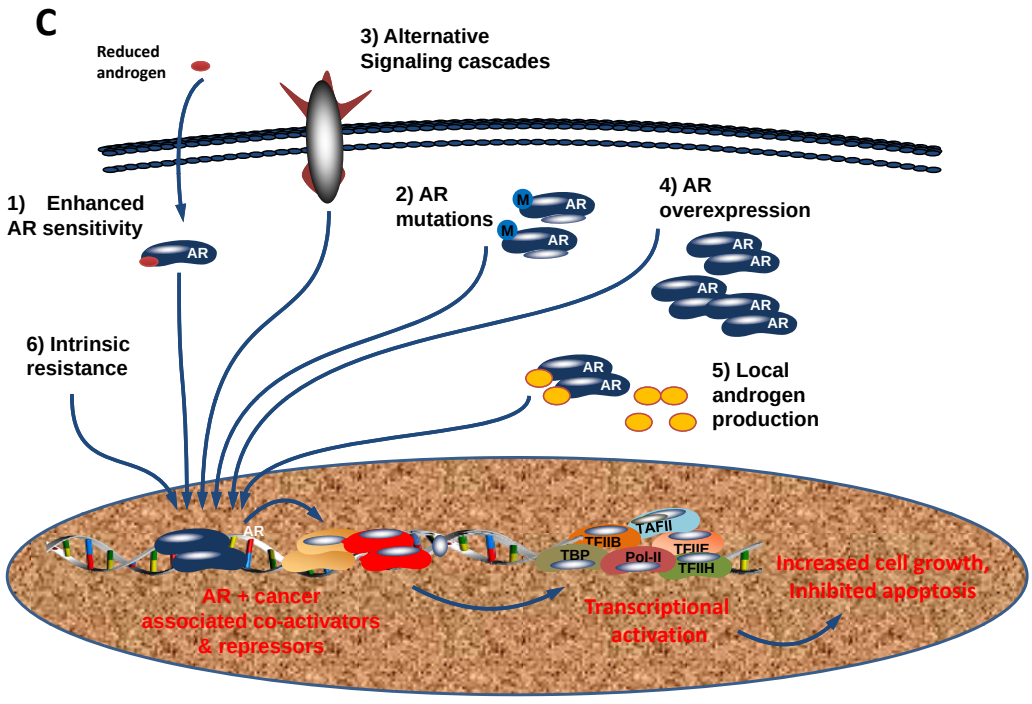

Fig. (3). Possible Mechanisms Driving the Hormone Refractory Phenotype. The hormone refractory phenotype is believed to be mediated by six possible mechanisms: 1) enhanced AR sensitivity, 2) AR mutations, 3) alternative signaling pathways, 4) AR overexpression, 5) local androgen production and 6) intrinsic cell resistance (see text for details). The functional consequences of all of these mechanisms will ultimately be conferred by the temporal and spatial recruitment of specific AR transcriptional complexes to the promoters of AR regulatory target genes.

Crucially only a limited number of co-regulatory proteins and are believed to co-regulate AR transcriptional activity during prostate progression [11]. Binding specificity at the promoter is conferred through a complex series of protein-DNA and protein-protein interactions with a multitude of co-activator and/or co-repressor proteins, which often include other transcription factor species [11, 12]. Numerous intricate layers of control such as alterations to intra- and extracellular signaling cascades, co-factor binding species, post-translational modifications, and alterations to protein/DNA conformation will also define the AR promoter complex. Such layers of control will not only define the repertoire of AR on specific target regulatory elements, but will also confer functional specificity and the magnitude of transcriptional activation or repression. This therefore is a critical focal point of regulatory crosstalk that controls aberrant AR target gene expression in prostate cancer which may be exploited for therapeutic gain in the treatment or inhibition of HSPC and HRPC. A growing body of evidence suggests that the ETS family of transcription factors may play a critical role in AR mediated transcriptional regulation in cancer.

ETS TRANSCRIPTION FACTORS AND CANCER

The ETS family of transcription factors have emerged as critical regulators of gene expression during cancer progression in most solid tumors and Leukemia's and are associated with hormonal mediated regulation. ETS factors are one of the largest families of transcriptional regulators with currently 27 family members [13-15]. They have diverse functions in almost all biological processes including development, proliferation, migration, invasion, and angiogenesis. An ever growing body of evidence demonstrates the importance of ETS genes in human carcinogenesis and is supported by the observations that ETS factor family members have aberrant expression patterns, regulate expression of multiple cancer associated genes, are chromosomally amplified or deleted, and are located at translocation breakpoints in leukemia's and solid tumors [13]. The biological and functional activity of ETS transcription factors and their role in cancer has been recently detailed in several recent reviews [13-16]. Evidence suggests that ETS factors act in concert to both positively and negatively regulate the pathways that control progression to metastatic cancer in prostate and breast tissues [14, 15, 17]. This reciprocal relationship has best been demonstrated for two ETS factors in particular, ETS1 and Prostate derived ETS factor (PDEF) (Watson *et al.*, this issue).

ETS1 levels are increased in epithelial tumors, leukemia's, astrocytomas and sarcomas [13, 15]. Its protein levels are significantly increased in clinical and latent prostate cancer relative to benign prostatic hyperplasia and normal prostate and are a proposed marker of poor prognosis [18]. Functionally, re-expression of ETS1 in non-invasive breast and prostate cancer cell lines enhances matrix degradation, cell growth, migration and invasion and promotes a pro-metastatic phenotype [19, 20]. This is mediated through the increased expression of genes including, but not limited to, BCL2, inhibitor of apoptosis (IAP's) family members, matrixmetalloproteinases (MMP's), urokinase plasminogen activator (uPA), and vimentin and the downregulation of E-cadherin [14, 21]. PDEF is an epithelial specific ETS family member and in contrast to ETS1 is associated with the negative regulation of metastatic potential [22-26]. Although PDEF message is sometimes found to be expressed in primary prostate and breast cancer and has been proposed as a prognostic marker [27], our research has recently identified post-translational regulation by microRNA's as a mechanism of PDEF protein loss during tumorogenesis in both tissues [28] (manuscript in prep). Increased PDEF expression in metastatic prostate and breast cell lines is associated with decreased metastatic potential through the inhibition of cell growth, migration and invasion [22, 24-26] through the decreased expression of cancer–associated genes such as uPA [25], survivin [23], vimentin, and SNAI2 [24] as well as the increased expression of Maspin [22] and E-cadherin [24]. Similarly to prostate, studies demonstrate that PDEF protein is expressed in normal breast tissue, but reduced in well-differentiated ductal carcinoma and lost in poorly differentiated ductal carcinoma [29]. PDEF protein loss is also associated with a more aggressive subset of estrogen receptor-negative, progesterone receptor-negative breast tumors [29].

ETS-AR Activity in Prostate Cancer

Increasing published data associates ETS transcription factors with prostate cancer progression and with AR transcriptional regulation. Molecular concept mapping is an analytical tool for exploring a network of relationships among a collection of biologically related gene sets (molecular concepts). Such analysis linked to over 14,000 molecular concepts has identified that increased ETS factor expression and AR transcriptional activity are critical transition points for prostate cancer progression (summarized in Fig. **4**) [30]. The increased transcriptional activity of pro-metastatic ETS family members was identified as the most

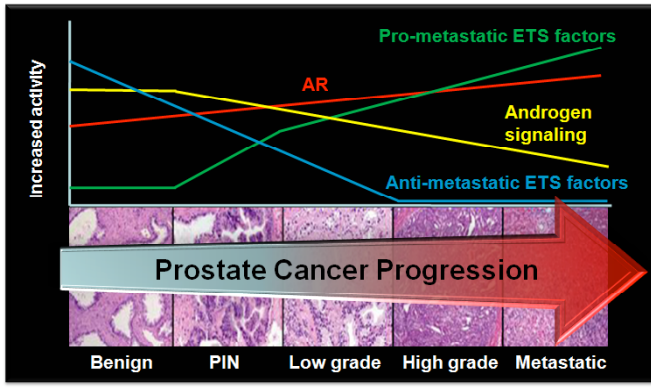

Fig. (4). Model of ETS-AR Protein Expression Profiles Observed during Prostate Cancer Progression. Molecular concept modeling and literature mining identify correlations between AR and ETS expression and prostate cancer progression. As prostate cancer progresses the transcriptional activity of pro-metastatic ETS factors is increased as the activity of anti-metastatic ETS factors are decreased. This correlates with an increase in overall AR levels but a decrease in androgen signaling activity (adapted from [1]).

frequent genetic alteration in prostate cancer and a critical molecular factor in mediating the transition from benign

epithelium to prostate intraepithelial neoplasia (PIN) (ETS1, ELK1 and GABPA), and the transition from PIN to prostate cancer (ERG, ETV1 and ETV4) [30]. The same study also identify that while overall AR levels increase during prostate cancer progression, overall androgen signaling is reduced indicating an alternative mechanism of AR transcriptional regulation in HRPC. Published evidence suggests that concurrent with the increased activity of pro-metastatic ETS transcription factors is the decreased activity of the anti-metastatic ETS factor PDEF (Fig. **4**) [14, 15, 17].

ETS-AR Transcriptional Co-Regulation

The importance of the functional interactions between ETS transcription factors and the AR has only recently been highlighted. ChIP-on-chip couples chromatin immunoprecipitation (ChIP) with high density tiling microarrays and can potentially identify the full regulome of any transcription factor [31]. Such analysis examining AR promoter binding in androgen sensitive LNCaP cells has identified over 1500 potential AR regulatory binding sites including the 15-bp ARE (AGAACAnnnTGTACC) inverted and direct repeat consensus sequence as well as two novel 6-bp "half-sites" that resemble one half of the 15bp consensus sequence (AGAACC and ACGAAC) [31]. Significantly, analysis of the AR bound sequences demonstrated that around 70% of the AR promoter binding sites were adjacent to sequence motifs for ETS transcription factors (GGAAAC and GGAACT) [31]. Furthermore, the promoter occupancy of ETS1 was defined for the AR bound promoter regions for CCNG2 and UNQ9419 and was functionally associated with their enhanced transcriptional activation. The antagonist mediated stimulation of AR activity in LNCaP cells resulted in the recruitment of AR and ETS1 to the nucleus and enriched ETS1 occupancy at the CCNG2 and UNQ9419 promoters [31].

ETS-AR transcriptional co-regulation has been demonstrated for several cancer-associated genes. PDEF regulates the PSA promoter both in an androgen dependent and independent manner [32]. PDEF directly associates with the DNA binding domain of the AR to regulate PSA promoter activation. In a yeast two-hybrid screen, overexpression of the AR co-regulatory factor NKX-3.1 identified PDEF as a direct binding partner and resulted in the suppression of PSA expression in LNCaP prostate cancer cells [33]. The overall contribution of PDEF-AR or PDEF-NKX-3.1 mediated regulation of PSA expression on prostate cancer progression has not yet been elucidated. The serpin peptidase inhibitor Maspin is a type II tumor suppressor gene expressed in normal breast and prostate epithelia [34]. The expression of Maspin in normal prostate cells is differentially regulated at the transcriptional level by a positive EBS and negative HREs. However in prostate cancer cell lines only the negative repression of the HRE by AR is observed as ETS1 regulation is inactive [35]. This indicates that the loss of Maspin expression in tumors may result from the loss of positive ETS activation in the presence of negative AR repression. Indeed in metastatic breast cancer cell line models, luciferase reporter assays demonstrate that increased expression of the anti-metastatic ETS factor PDEF results in the increased expression of Maspin resulting in decreased cell growth, migration and invasion [22]. This interaction was specific for PDEF as neither FLI1 nor ETS1 were able to activate the promoter. Survivin is a member of the inhibitor of apoptosis family and is associated with prostate cancer progression and the drug resistant phenotype. In hormone sensitive prostate cancer cells, but not hormone refractory, AR agonist (DHT) increases and androgen antagonist (Flutamide) decreases Survivin expression [36]. Dominant negative inhibition of Survivin activity increased the efficacy of AR antagonist treatment. In prostate cancer cells, the survivin promoter is directly occupied by PDEF resulting in the negative regulation of survivin expression resulting in decreased metastatic potential (Findlay *et al.*, Submitted).

MMPs are zinc-dependent endopeptidases with many biological roles including cell proliferation, migration and differentiation [37]. Several MMPs are implicated in many aspects of cancer progression. Both interstitial collagenase (MMP1) and matrilysin (MMP7) are negatively co-regulated by AR. This negative regulation is dependent upon the binding of the ETS family member ERM at an EBS in the MMP1 promoter [38]. Interestingly this interaction does not require AR DNA binding but does require the N-terminal region of AR. This interaction was specific for AR as it was not observed in the presence of other hormone receptors. ETS factors are known to also positively regulate MMPs. Up-regulated ETS1 and ETS2 increases the expression of both MMP1 and MMP9, which are associated with clinical features such as lymph node status and prognosis [19].

Up to 80% of prostate cancers have gene fusions containing an androgen dependent 5' genomic regulatory element (TMPRSS2) fused to an ETS family member (ERG, ETV1, ETV4, ETV5) and have been the focus of many review articles [39-42]. Furthermore, ETV1, ETV4 and ETV5 are associated with gene fusions with other androgen regulated genes including SLC45A3, 22Q11.23, CANT1, KLK2 and HERV-K [39, 42]. Unlike ERG, *ETV gene fusions* exhibit a large degree of variability as 12 unique 5' structural fusion partners have been identified [40]. While there are conflicting data as to the clinical implications of ETS fusions the more in depth studies point towards a poorer prognosis and poor survival especially involving the duplication of re-arranged ERG [40]. The hormonal regulation of ETS genes fusions is believed to result in the over-expression of full length and/or truncated oncogenic ETS factors, particularly ERG and ETV1. When expressed in the prostate of adult mice the elevated expression of either ERG or ETV1 induces hyperplasia and PIN lesions and when combined with other prostate cancer associated genetic alterations leads to an aggressive phenotype [43]. Intriguingly, overexpression of AR only produced hyperplastic lesions in the presence of increased levels of ERG and promoted invasive carcinoma. Recently, an ETS factor family member has been shown to be directly regulated by the AR in an androgen dependent manner [44]. ELK4 expression is elevated in prostate cancer and significantly increased in HRPC tissues. An ARE situated 467 base pairs from the ELK4 transcriptional start site was shown to be critical for ELK4 transcriptional activation in response to androgen treatment in prostate cell lines.

A major challenge to the prolonged efficacy of chemotherapy in HRPC is the acquisition of a drug resistant

phenotype [45]. Several molecular mechanisms can confer drug resistance and these include increased drug efflux, DNA repair and drug de-toxification as well as apoptosis insensitivity [46]. The published literature to date has identified a significant role for apoptosis inhibition in mediating resistance in HRPC and its re-activation is crucial for the successful treatment of prostate cancer. Significantly, ETS and AR are both associated with the transcriptional regulation of apoptosis: Expression of antisense ETS2 or dominant negative (DN)-ETS2 inhibits anchorage independent growth of prostate cancer-derived cells [13]. Furthermore, direct inhibition of ETS2 transcription by triplex forming oligonucleotides inhibits cell growth and activates apoptosis in human prostate cancer cell [47]. The co-expression of retinoblastoma with AR in DU-145 prostate cancer cells results in apoptosis activation through mitochondria damage-initiated caspase activation [48]. While AR ligand promotes apoptosis inhibition in hormone sensitive LNCaP cells, irradiation of the same cells with visible light caused apoptosis which required the formation of 1,2,3,4-tetrahydro-2,2-dimethyl-6-(trifluoromethyl)-8-pyridono[5,6-g]quinoline (TDPQ)/AR complexes [49].

ETS-AR Transcriptional Co-Regulatory Factors

Over 200 nuclear receptor co-regulatory proteins have already been identified which comprise of proteins involved in almost all cellular processes including chromatin remodeling, histone modification, DNA repair, sumoylation, RNA metabolism, signal transduction and cell cycle regulation. As detailed by Heemers & Tindall [11], transcriptional co-regulators can be divided into three broad categories: 1 – general transcriptional complex proteins, 2 – co-activator or co-repressor proteins and 3 – transcription factors. Critically, while there is a myriad of AR transcriptional co-regulators it is believed that only a defined subset of those co-factors promote the aberrant formation of AR transcriptional complexes at the promoters of cancer associated genes [11]. ETS factors also require functional and physical interactions with co-regulatory proteins for their transcriptional activity [20, 50, 51]. Although not studied to the extent of the AR, several ETS associated transcriptional factors have been identified [20, 50, 51]. For example, combinatorial control has been shown to be a major characteristic of ETS gene regulation through synergistic interactions with other key transcription factors at composite consensus sites (ETS/AP1 (Fos/Jun), ETS/AML1 (Runx), ELF1/NFκB, ETS1/Pax5, and ETS/SRF) [51, 52]. Among the first characterized ETS interactions were studies demonstrating functional co-operativity between ETS factors (PEA3, ETS1, ERG) and the AP1 transcriptional complex in the up-regulation of uPA, MMP1, GM-CSF and TIMP1, all known mediators of cancer progression [51, 52]. Interestingly, the AP1-like protein MafB inhibits the ETS1 transactivation mediated by AP1/EBS [53]. A review of the literature demonstrates that several transcriptional co-regulatory proteins have known associations with both ETS and AR transcriptional activity during tumorogenesis (Table **1**). The transcriptional co-regulator NKX3.1 functionally interacts with both AR and PDEF to regulate the PSA gene promoter [31, 32]. The p160 steroid receptor co-activator family members SRC1 and SRC3 are also associated with both AR and ETS transcriptional regulation. SRC1 is overexpressed in 50% of HSPC and over 60% of HRPC patients [54]. SRC3 expression correlates with prostate cancer grade and stage as well as decreased disease-free survival [54]. In breast cancer tumor and immortalized cell cultures, ETS1 and ETS2 growth factor induction stimulated the recruitment of SRC1, SRC3 and NCoR demonstrating that steroid receptor co-activators also interact with non-steroidal transcription factors [55]. Table **1** lists other co-regulatory proteins that are independently associated with both ETS and AR transcriptional activity that have not yet been shown to be involved in ETS-AR transcriptional co-regulation. Further research is required to identify a possible role for these factors in mediating ETS-AR transcriptional co-regulation specifically.

FUTURE RESEARCH DIRECTIONS

In prostate cancer, one challenge facing basic scientists and clinicians is to develop a greater understanding of the molecular biology augmenting the androgen refractory phenotype in order to develop new therapeutic regimes that either prevent its occurrence or circumvent its effects. Targeting the AR through ADT was first recognized as a treatment for HSPC over 50 years ago and much is known about its effects on AR transcriptional regulation. Critically, AR is now known to continue to play a critical role as prostate cancer progresses through HSPC and HRPC (Fig. **4**) [30, 56] and recent research has led to the design of treatment regimes for HRPC [7]. However, resistance to ADT in HSPC, and the absence of a truly effective treatment for HRPC highlights an urgent need for new strategies for their treatment. With this in mind, very little is known about the transcriptional mechanisms that mediate progression through the hormone sensitive to hormone refractory phenotypes.

As outlined in this review, increasing published data associates ETS transcription factors with prostate cancer progression and with the transcriptional activity of the AR:

- Around seventy percent of HRE's are adjacent to ETS binding sites [31];
- ETS-AR transcriptional co-regulation has been demonstrated for a subset of these AR promoters [31];
- Molecular concept mapping identifies AR and ETS factor transcriptional activity as critical transition points for prostate cancer progression [30];
- AR and ETS expression is associated with both the positive and negative regulation of progression in prostate cancer [15, 76];
- Up to 80% of prostate cancers have gene fusions containing an androgen dependent 5' genomic regulatory element (TMPRSS2) fused to an ETS family member (ERG, ETV1, ETV4, ETV5) [39].
- ETS-AR transcriptional complexes co-occupy the promoters of prostate cancer associated genes to regulate their expression [32, 77].

Given the critical roles both ETS factors and the AR play in the development and progression of prostate cancer, mechanistic insight into their transcriptional regulation of

Table 1. Co-Regulatory Proteins Independently Associated with both ETS and AR Transcriptional Activity

Co-factor	Biological function	Associated ETS factor	ETS associated function	AR associated function
AP-1	Transcription factor	ETS1	Regulate gene transcription [20, 57]	Synergistically regulate gene transcription [58]
CBP	Histone modifier	ETS1/ERG1	Promotes transcriptional activation [50]	Regulates AR transcriptional activity [11]
DAXX	Death domain protein	ETS1	Represses transcriptional activity [59]	Negatively regulates AR [60]
ER	Nuclear receptor	ETS1	Ligand independent co-regulation [20]	Direct promoter interactions [61]
FLNA	Cytoskeleton organization	PDEF	Proteomic analysis associated [62]	Regulates AR and co-regulatory proteins [11]
GATA1	Transcription factor	FLI1	Ligand independent co-regulation [50, 63]	Regulates AR co-regulatory factors [64]
GATA3	Transcription factor	ELF1/ETS1/ETS2	Direct promoter interactions [20, 63]	Regulates AR/co-regulatory proteins [11]
NCoR	Receptor co-regulator	ETS1/TEL/ETS2	ETS transcriptional repression [55]	Nuclear receptor co-regulatory protein [65]
NKX3.1	Transcriptional co-factor	PDEF	Regulate androgen associated genes [32, 33]	Regulate androgen associated genes [66]
OCT1	Transcription factor	PU-1/ETS1	Direct promoter interactions [67]	AR Transcriptional regulation [11]
p53	Transcription factor	ETS1	p53 dependent apoptosis [20]	p53 negatively regulates AR [11, 68]
PIASy	Sumoylation	ETS1	Sumylation independent transcription [56]	Sumylation independent transcription [69]
SENP1	Sumoylation	ETS1	Desumoylates Ets-1 [56]	Enhances AR transcriptional activity [70]
SRC1	Histone modifier	PEA3/ETS1/ETS2	Transcriptional activator [55, 71]	AR transcriptional co-activator [54]
SRC3	Histone modifier	ETS2	Ets-2 regulation of myc [72]	AR transcriptional co-activator [54]
SUMO1	Sumoylation	ETV6/ETS1	ETS ubiquitination [73, 74]	Direct AR transcriptional regulator [70]
UBC9	Sumoylation	ETS1/ELK1	Regulates transciptional activity [56, 75]	Receptor dependent transcription [11]

HSPC and HRPC will be provided by determining the contribution of ETS, AR and ETS-AR mediated transcriptional control to each phenotype. This would greatly impact our biological understanding of the etiology of prostate cancer as it progresses to its most lethal stage. The development of novel therapeutics that target ETS or ETS-AR mediated regulation of androgen dependency will directly impact prostate cancer mortality as onset of HRPC drastically reduces median survival rates [5, 7]. Direct targeting of the AR or the ETS factor family as a whole, is likely to be toxic to normal cells. By targeting defined interactions between AR and specific ETS family members, we would expect to significantly reduce off target effects and treatment toxicity and at the same time increase the efficacy of treatment. The overall lack of therapeutics that produces a sustained inhibition of AR transcriptional activity highlights the requirement for modulators of AR signaling across the full range of AR biology. The binding interface between AR and its co-regulatory proteins is an attractive target for therapeutic intervention. While targeting protein-protein interactions has its challenges, our in depth knowledge of both the overall structure and functional domains of AR [78] and specific ETS family members, including PDEF [79] and ETS1 [80] makes targeting their interactions particularly attractive for drug discovery.

An increased understanding of AR transcriptional regulation during the progression to HRPC will impact our understanding of prostate cancer therapeutic outcomes and regimes by:

- Confirming a role for ETS-AR transcriptional regulation in mediating progression to HRPC;
- Establishing a unique concept for investigating transcriptional co-regulation in prostate cancer;
- Defining a mechanism by which multiple transcription factors can reciprocally regulate cancer associated target genes during prostate cancer progression;
- Identifying novel therapeutic aspects for prostate cancer treatment.

In the shorter term, the identification of multiple target genes that mediate the ETS and/or ETS-AR transcriptional response may identify novel prognostic markers that may

more accurately predict outcome. Due to the heterogeneity of prostate cancer, multivariate outcome prediction models cannot reliably predict true prognosis. Therefore, new and more accurate and reliable predictive markers are urgently required to be able to separate patients with more benign tumors requiring less aggressive treatment, from those with more potentially lethal tumors requiring more radical therapy. If specific patterns of target gene expression are required for the androgen sensitive and refractory phenotypes, their identification may provide a molecularly based profile that defines poor prognosis signatures and may form selection criteria for the appropriate treatment of prostate cancer patients. Finally, promising prognostic markers can be further investigated in future proposals in order to gain mechanistic insight into their function and if suitable be brought back to the clinic as novel diagnostic markers.

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