Androgen receptor molecular biology and potential targets in prostate cancer

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***Abstract*:** The androgen receptor (AR) is a key transcriptional regulator and therapeutic target in prostate cancer. During androgen deprivation therapy to treat metastatic prostate cancer, surviving cells acquire increased AR signaling through a variety of mechanisms, one of which is enhanced interactions with AR coactivators. One recently identified AR-specific coregulator expressed only in human and nonhuman primates is the melanoma antigen gene protein-A11 (MAGE-11). MAGE-11 increases AR transcriptional activity through direct interactions with AR and other coactivators, and its levels increase during prostate cancer progression to castration-recurrent growth. The MAGE-11 gene is located at Xq28 on the human X chromosome as part of an X-linked MAGE gene family of cancer–testis antigens. MAGE-11 stabilizes AR when androgen levels are low, and functions in a transcriptional hub to promote AR-mediated gene activation. The evolutionary development and organization of the MAGE-11 gene within the cancer–testis antigen family suggests that MAGE-11 provides a gain-of-function to AR among primates in both normal physiology and cancer, and may serve as a therapeutic target in the treatment of advanced prostate cancer.

***Keywords*:** androgen receptor, cancer–testis antigens, MAGE-11, MAGE-A11, N/C interaction, X chromosome, X-linked genes

Introduction

Prostate cancer develops initially as an androgen-dependent disease that relies on the androgen receptor (AR) for growth and progression. The androgen dependence of prostate cancer reflects the properties of the normal prostate gland and explains the initial effectiveness of androgen deprivation therapy as a first-line therapy. However, resistance to androgen deprivation develops with time and prostate cancer cells undergo recurrent growth despite low levels of circulating androgen. Relapsed prostate cancer, known as castration-recurrent or castration-resistant prostate cancer, is associated with high mortality due to the lack of effective long-term treatment strategies. Castration-recurrent prostate cancer is a complex disease that develops during the course of androgen deprivation therapy and maintains a dependence on AR for its metastatic potential.

One major goal of prostate cancer research is to understand the mechanisms by which AR signaling promotes castration-recurrent prostate cancer growth so that new treatments can be developed to prevent or treat relapse of the disease. Although research has progressed rapidly in the field, there has been a lack of therapies that provide a significant survival benefit. Many aspects of prostate cancer research have been summarized in recent reviews that address a range of topics related to AR signaling and clinical management of the disease. These include the development of inhibitors that target steroid metabolic pathways [Lassie and Dawson, 2010; Reid *et al.* 2010; Attard *et al.* 2009] based on evidence that castration-recurrent prostate cancer cells acquire the ability to synthesize androgen [Locke *et al.* 2008; Montgomery *et al.* 2008; Stanbrough *et al.* 2006; Titus *et al.* 2005b; Mohler *et al.* 2004], therapeutic and vaccine approaches [Cha and Fong, 2010; Stavridi *et al.* 2010; Chi *et al.* 2009; Vis and Schröder, 2009], molecular mechanisms that underlie prostate cancer progression [Dutt and Gao, 2009], and the role of AR mutations [Brooke and Bevan, 2009], epigenetic mechanisms [Schulz and Hoffmann, 2009] and AR coregulators in prostate cancer progression [Golias *et al.* 2009; Heemers and Tindall, 2007]. The present review focuses on molecular aspects of AR function in relation to the AR

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selective coregulator, melanoma antigen gene protein-A11 (MAGE-11).

AR signaling in prostate cancer

AR is a member of the steroid/nuclear receptor superfamily of ligand-dependent transcription factors and acts in the nucleus to regulate androgen-dependent gene expression in response to the two active androgens, testosterone or dihydrotestosterone (DHT). Androgen binding induces a conformational change in AR that is associated with the androgen-dependent AR NH_2- and carboxyl-terminal (N/C) interaction, the recruitment of coactivators, and stabilization of a multiprotein transcription complex involved in chromatin remodeling. AR binds to specific DNA androgen response elements associated with androgen-regulated genes. Loss-of-function AR gene mutations that cause the androgen insensitivity syndrome demonstrate that AR is required to mediate the effects of androgen. AR DNA binding domain mutations that cause androgen insensitivity demonstrate that AR must bind DNA in the nucleus.

Under normal physiological conditions, AR function depends on high affinity binding of testis-derived androgen. However, in castration-recurrent prostate cancer cells, AR is no longer dependent on circulating androgen and may be activated by androgen synthesized locally. Evidence suggests that AR transcriptional activity is a major factor in the growth of androgen-dependent and castration-recurrent prostate cancer. This is evident from studies in which prostate cancer cell growth is arrested by lowering AR levels using small inhibitory RNAs [Ponguta *et al.* 2008; Li *et al.* 2007; Guo *et al.* 2006; Yuan *et al.* 2006; Furutani *et al.* 2005; Zegarra-Moro *et al.* 2002]. The proposed mechanisms for increased AR signaling in castration-recurrent prostate cancer include local prostate cancer tissue androgen production [Titus *et al.* 2005b; Mohler *et al.* 2004], AR gene amplification [Linja *et al.* 2001; Visakorpi *et al.* 1995], increased mitogen signaling [Culig *et al.* 2005; Gregory *et al.* 2004], increased levels of AR coactivators [O'Malley, 2009; Gregory *et al.* 2001], increased sensitivity to low androgen levels [Waltering *et al.* 2009; Gregory *et al.* 2001; Klein *et al.* 1997] and the expression of AR somatic mutants. The identification of sufficient levels of androgen in castration-recurrent prostate cancer cells to activate AR has challenged the notion that AR can be activated independent of androgen binding. Some studies suggest intratumor testosterone or DHT produced through the metabolic conversion of adrenal androgens, which do not activate wild-type AR, or the *de novo* synthesis of active androgens from cholesterol, can activate AR when circulating testicular testosterone is undetectable. On the other hand, there is *in vitro* evidence that AR can be transcriptionally activated in the absence of androgen through mechanisms that include enhanced mitogen signaling and interactions with coactivators.

Somatic AR mutations are uncommon in prostate cancer, but occur with greater frequency in more advanced stages of the disease. The gain-in-function associated with these mutations exemplifies the ability of prostate cancer cells to adapt to androgen deprivation or antiandrogen therapy and maintain AR function. Unlike naturally occurring loss-of-function AR germline mutations that cause incomplete masculine development in 46XY genetic males with the androgen insensitivity syndrome [Quigley *et al.* 1995], somatic AR mutations that develop in prostate cancer more often increase AR responsiveness to adrenal androgens, other steroids and AR antagonists. Depending on the location and nature of the amino acid mutation, AR signaling can be increased by binding other steroids [Askew *et al.* 2007; He *et al.* 2006; Chang *et al.* 2001; Tan *et al.* 1997; Harris *et al.* 1991; Veldscholte *et al.* 1990]. The gain-of-function AR somatic mutations that occur under the selective pressure of androgen deprivation within the genetically unstable environment of cancer may be further facilitated by the single allele status of the AR gene on the human male X chromosome.

Primate-specific cancer–testis antigens in the MAGE gene family

The single allele human male X chromosome has been subject to selective pressure during the evolution of primates that has resulted in the accumulation of male-advantage genes involved in sex development and reproduction [Delbridge and Graves, 2007; Saifi and Chandra, 1999]. In addition to the AR gene at Xq11-12 [Brown *et al.* 1989], there are X-linked germ cell specific genes required for male reproductive function [Zheng *et al.* 2010], and a group of X-linked cancer–testis antigen genes whose function has been associated with spermatogenesis. Of the 153 cancer–testis antigen genes [Almeida *et al.* 2009], 83 cancer–testis antigens occur within multigene families and represent ~10% of the genes on the human X chromosome

[Ross *et al*. 2005; Simpson *et al*. 2005]. One class of cancer–testis antigen is the melanoma antigen gene (MAGE) family that has 52 members. MAGE genes were named based on their initial identification in melanoma and have been divided into eight subclasses, MAGE-A through H, whose functions are mostly unknown. Members of the MAGE family share a conserved ~200 amino acid MAGE homology domain in the carboxyl-terminal region [Barker and Salehi, 2002; Chomez *et al*. 2001]. The AR specific coregulator MAGE-A11 (MAGE-11) is one of 12 members of the MAGE-A subfamily of cancer–testis antigen genes located in the Xq28 region of the human X chromosome [Rogner *et al*. 1995; De Plaen *et al*. 1994]. The MAGE homology domain in human MAGE-11 includes amino acid residues 222 to 421 in the 429 amino acid full-length protein [Bai and Wilson, 2008].

Similar to other cancer–testis antigen genes, MAGE genes have undergone species-specific expansion through gene duplication by retrotransposition from the MAGE-D subfamily that rapidly diverged among mammals [Chomez *et al*. 2001]. Some MAGE genes are conserved between mouse and man, such as the gene that codes for Necdin, a cell cycle regulatory protein. Deletion of the Necdin gene results in the neurogenetic disorder known as Prader-Willi syndrome [Lee *et al*. 2005]. However, many MAGE genes including MAGE-11 are poorly conserved through evolution, suggesting they evolved more recently through retrotransposition. The MAGE-11 gene arose by gene amplification within the primate lineage [Delbridge and Graves, 2007] and is expressed only in human and nonhuman primates. Thus, while some human MAGE genes have homologues in mice [De Plaen *et al*. 1999], the species-specific expansion of the MAGE gene family resulted in MAGE-11 being unique to primates. It is noteworthy that primates have a large number of retroposed insertions that have contributed to the evolution of new functions [Baertsch *et al*. 2008]. The more recent evolution of genes involved in reproduction and fertilization is associated with speciation [Turner and Hoekstra, 2008], and primate-specific genes are preferentially expressed in tissues of the reproductive tract [Tay *et al*. 2009].

Genes in the MAGE-A subfamily were first thought to be expressed mainly in cancer and code for ~300 amino acid residue proteins from a single exon that included the MAGE homology domain [Artamonova and Gelfand, 2004; Barker and Salehi, 2002; Chomez *et al*. 2001; Jurk *et al*. 1998; Rogner *et al*. 1995]. However, the MAGE-11 gene has four coding exons in the human Xq28 chromosomal region. The first of the three short coding exons contains a nuclear targeting signal consistent with the predominantly nuclear localization of MAGE-11 [Bai and Wilson, 2008; Irvine and Coetzee, 1999]. Based on the prevalence of retroposed elements in the primate lineage, the three 5' upstream coding exons of the MAGE-11 gene most likely derived from the splicing of mRNA-derived retrocopies that amended the function of the MAGE-A subfamily and enabled MAGE-11 to be an AR coregulator. This is supported by evidence that deletion of the MAGE-11 NH_2-terminal region causes loss of its AR coactivator function. The MAGE-11 protein is 429 amino acid residues in length and migrates as a 60–65 kDa protein on denaturing gels. Unlike some MAGE genes that are expressed primarily in cancer with the exception of male germ cells, MAGE-11 is expressed in both normal reproductive tissues and cancer [Irvine and Coetzee, 1999; Rogner *et al*. 1995].

The physiological function of most cancer–testis antigens remains unknown [Simpson *et al*. 2005; Scanlan *et al*. 2004]. However, several MAGE genes have been shown to be involved in cell cycle progression, neural development and apoptosis. For example, p53 function is modulated by hNRAGE (MAGE-D1), Necdin (MAGE-L2) and MAGE-A2, all members of the MAGE gene family [Monte *et al*. 2006; Wen *et al*. 2004; Taniura *et al*. 1999]. MAGE-D1 interacts with proteins involved in cell cycle control and apoptosis [Barker and Salehi, 2002], and regulates the cell cycle by enhancing p75 neurotrophin-mediated apoptosis [Salehi *et al*. 2000]. Necdin is a neuron-specific growth suppressor that interacts with cell growth promoting proteins that include viral oncogenes [Ohman Forslund and Nordqvist, 2001; Taniura *et al*. 1999]. Necdin facilitates cell cycle exit and cell survival [Matsumoto *et al*. 2001], and its loss results in the rare neurogenetic disorder known as Prader–Willi syndrome [Lee *et al*. 2005; Boccaccio *et al*. 1999]. The entire Necdin MAGE homology domain was required for the interaction with p53 [Taniura *et al*. 2005]. MAGE-A2 acts as a p53-histone deacetylase 3 assembly protein that provides a survival

advantage to cancer cells [Monte *et al*. 2006]. Suppression of class I MAGE-A, B and C members coded on the human-X-chromosome-induced apoptosis and the acetylation of p53 in melanoma cell lines, suggesting that MAGE proteins promote tumor survival [Yang *et al*. 2007]. MAGE-11 shares sequence similarity with the adenovirus early region 1A viral oncoprotein E1A that binds p300, a potent acetyltransferase and cell cycle regulator. This suggests that MAGE-11 in association with AR functions in cell cycle regulation and provides a growth advantage to prostate cancer cells.

MAGE-11 as AR coregulator

More than 50 coactivator proteins are reported to interact with AR and the levels of some of these proteins increase during prostate cancer progression [Heemers and Tindall, 2007]. AR transcriptional activity depends on interactions with coregulator proteins mediated primarily through two activation domains. Activation function 1 (AF1) is in the AR NH_2-terminal region, and activation function 2 (AF2) is a hydrophobic surface of the highly structured carboxyl-terminal ligand-binding domain. The p160 coactivators, named for their molecular weight of ~160 kDa and referred to as SRC1, SRC2/TIF2/GRIP1 or SRC3/AIB1/TRAM1/ACTR, interact principally with AF2 in the AR ligand-binding domain through the coactivator L*XX*LL motifs. Other AR coactivators that interact with the AR NH_2-terminal region are less well characterized. Increased levels of AR coactivators is one mechanism by which castration-recurrent prostate cancer may acquire enhanced AR signaling to promote tumor growth during androgen deprivation therapy.

The AR NH_2-terminal region contains the AR F*XX*LF motif sequence ^{23}FQNLF^{27} that interacts with AF2 as an amphipathic alpha helix to mediate the androgen-dependent AR N/C interaction [He *et al*. 2004, 2000]. The AR N/C interaction is required for the activation of many androgen-regulated genes [He *et al*. 2002, 2000]. Androgen-dependent binding of the AR F*XX*LF motif to AF2 can competitively inhibit binding of the p160 coactivator L*XX*LL motifs, which bind with lower affinity to AF2 than the AR F*XX*LF motif [Askew *et al*. 2007; He *et al*. 2001].

MAGE-11 was identified as an AR coactivator in a yeast two hybrid screen of a human testis library using an AR NH_2-terminal F*XX*LF motif fragment as bait [Bai *et al*. 2005]. The screen was based on the ability of mutations introduced into the AR F*XX*LF motif to expose AF2 and increase p160 coactivator L*XX*LL motif binding. It was postulated that a protein that binds the AR F*XX*LF motif might similarly relieve inhibition of AF2 caused by the AR N/C interaction. As predicted, through its interaction with the AR NH_2-terminal F*XX*LF motif, one mechanism by which MAGE-11 increases AR transcriptional activity is to expose AF2 for increased p160 coactivator recruitment [He *et al*. 2002, 2001].

The AR NH_2-terminal F*XX*LF motif therefore serves at least two functions. In addition to binding the AR AF2 site to mediate the androgen-dependent AR N/C interaction, the AR F*XX*LF motif binds MAGE-11. The competitive relationship between the AR N/C interaction and MAGE-11 binding modulates AR transcriptional activity. In the interaction with MAGE-11, the AR F*XX*LF motif binds a MAGE-11 F-box region that resembles the F-box in cyclin F for which it was originally named [Askew *et al*. 2009]. The F-box is a ~40 amino acid sequence that has a conserved spacing of hydrophobic residues. The presence of an F-box in MAGE-11 suggests that it functions as part of a ubiquitin ligase complex. The MAGE-11 F-box contains threonine 360 (Thr-360), which is phosphorylated by cell cycle checkpoint kinase Chk1 in response to epidermal growth factor signaling [Bai and Wilson, 2008]. Phosphorylation at Thr-360 signals the monoubiquitinylation of MAGE-11 at two lysine residues outside the F-box. Both phosphorylation and monoubiquitinylation of MAGE-11 are required to bind the AR F*XX*LF motif (Figure 1).

AR signaling is also influenced by the ability of MAGE-11 to stabilize AR when androgens levels are low. This may be an important mechanism to maintain AR levels in castration-recurrent prostate cancer. MAGE-11 also increases AR transcriptional activity through direct interactions with p160 coactivators and p300 (Figure 2) [Askew *et al*. 2010, 2009]. MAGE-11 functions synergistically with transcriptional mediator protein 2 (TIF2), a p160 coactivator, and with p300, a potent and ubiquitous transcriptional regulator with histone acetyltransferase activity.

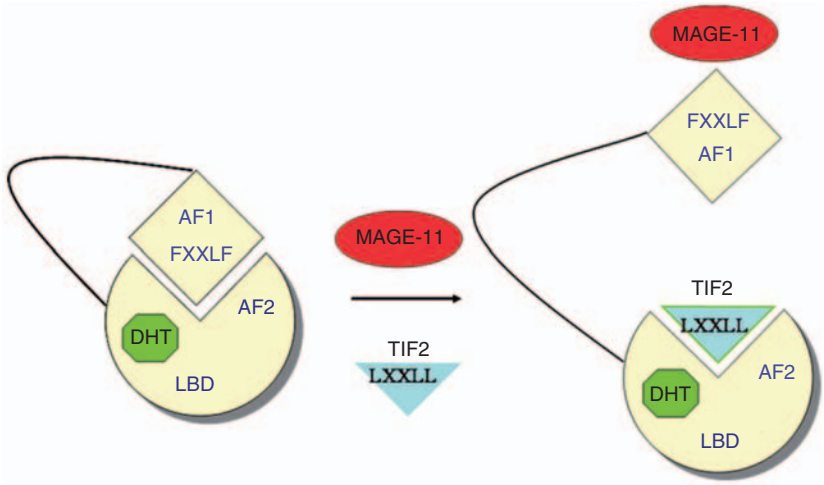

Figure 1. MAGE-11 binds the AR NH_2-terminal F*XX*LF motif and competes for the AR N/C interaction to increase AF2 binding of p160 coactivators and AR transcriptional activity. AR (yellow) bound to DHT (green) undergoes the N/C interaction between the AR NH_2-terminal F*XX*LF and AF2 in the ligand-binding domain. The AR F*XX*LF motif also binds MAGE-11 (red), which exposes AF2 for p160 coactivator TIF2 (blue) recruitment by L*XX*LL motifs and increases AR transcriptional activity. MAGE-11 also increases AR transactivation by bridging to other coregulatory proteins. AF1, activation function 1; AF2, activation function 2; AR, androgen receptor; DHT, dihydrotestosterone; LBD, ligand-binding domain; MAGE-11, melanoma antigen gene protein-A11; TIF2, transcriptional mediator protein 2.

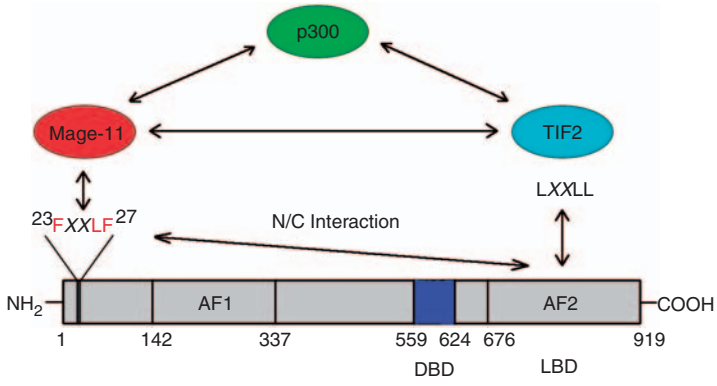

Figure 2. MAGE-11 increases androgen receptor (AR) transcriptional activity by interacting with TIF2 and p300. Binding of MAGE-11 to the AR NH_2-terminal F*XX*LF motif opens AF2 in the ligand-binding domain for p160 coactivator TIF2 binding. Direct interactions between MAGE-11, TIF2 and p300 provide additional mechanisms for increased AR signaling in prostate cancer. AF1, activation function 1; AF2, activation function 2; DBD, DNA-binding domain; LBD, ligand-binding domain; MAGE-11, melanoma antigen gene protein-A11; TIF2, transcriptional mediator protein 2.

In addition to being an AR-specific coregulator, MAGE-11 has been shown to interact with the hypoxia-inducible factor prolyl hydroxylase (PHD), a cytoplasmic enzyme that hydroxylates and thereby controls the stability of hypoxia-inducible factor-α (HIF-1α) [Aprelikova *et al.* 2009; Berra *et al.* 2003]. The inhibitory effect of MAGE-11 on PHD hydroxylase activity results in the stabilization of HIF-1α, a protein that allows tumors to adapt to the hypoxic conditions associated with low oxygen [Wang *et al.* 1995].

Regulated expression of MAGE-11 in normal tissues and prostate cancer

MAGE-11 is expressed predominantly in tissues of the human male and female reproductive tracts. Cancer–testis antigen gene expression was initially thought to be limited to human germ cells of the adult testis with limited expression in placenta and ovary, and overexpression in different types of cancer [Kalejs and Erenpreisa, 2005; Simpson *et al.* 2005; Scanlan *et al.* 2002]. However, MAGE-11 is expressed in normal

human testis, ovary, endometrium, adrenal gland and placenta [Bai *et al*. 2008, 2005; De Plaen *et al*. 1994] and in prostate cancer.

The MAGE-11 gene is regulated through at least two mechanisms. MAGE-11 expression is upregulated by cyclic AMP in human endometrial and prostate cancer cells [Karpf *et al*. 2009; Bai *et al*. 2008]. MAGE-11 levels increase in human endometrial epithelial cells after the LH surge during the early to mid secretory phase of the menstrual cycle with highest levels during the window of implantation [Bai *et al*. 2008]. This suggests that LH signaling through cyclic AMP is responsible for the increase in MAGE-11, and that MAGE-11 has a role in human embryo implantation. MAGE-11 mRNA levels are low in most normal tissues, but increase by ~50-fold in the endometrium epithelium during the window for implantation, and up to 1000 fold during prostate cancer progression to castration-recurrent growth [Karpf *et al*. 2009]. Thus, one mechanism for increased MAGE-11 expression and possibly other cancer–testis antigens in advanced forms of cancer may be increased cyclic AMP signaling. However, in DU145 prostate cancer cells that do not express MAGE-11, the MAGE-11 gene is fully methylated near the transcription start site and levels of MAGE-11 mRNA do not increase in response to cyclic AMP. This suggests that promoter DNA methylation can render the MAGE-11 gene refractory to stimulation by cyclic AMP.

A second predominant mechanism for increased cancer–testis antigen expression widely demonstrated in cancer is DNA hypomethylation. This is supported by evidence that the demethylating agent 5-aza-2-deoxycytidine increases cancer–testis antigen expression [Shichijo *et al*. 1996]. MAGE genes are normally repressed by DNA methylation and histone deacetylation [Wischnewski *et al*. 2006; De Smet *et al*. 1999] that may involve methyl-CpG binding proteins that recruit histone deacetylases [Wischnewski *et al*. 2007]. In agreement with these findings, the MAGE-11 promoter has a high CpG content that is subject to DNA methylation. The MAGE-11 gene promoter is progressively hypomethylated at the transcription start site during prostate cancer progression, and is partially to fully hypomethylated in castration-recurrent prostate cancer to an extent that correlates with MAGE-11 mRNA levels [Karpf *et al*. 2009]. In agreement with the higher expression of cancer–testis antigens in higher-grade cancers with poor prognosis [Andrade *et al*. 2008; Napoletano *et al*. 2008; Velazquez *et al*. 2007; Gure *et al*. 2005], MAGE-11 levels were higher in castration-recurrent prostate cancer. During the transition to castration-recurrent growth, MAGE-11 mRNA levels increase to a greater extent than AR mRNA [Karpf *et al*. 2009], suggesting that stabilization of AR by MAGE-11 is one mechanism to promote prostate cancer progression.

The increased expression of MAGE-11 by DNA hypomethylation in castration-recurrent prostate cancer may reflect genomewide DNA hypomethylation, an epigenetic event associated with cancer [Ehrlich, 2002; De Smet *et al*. 1996]. Although global hypomethylation was reported for colorectal cancer without increased cancer–testis antigen expression [Park and Lee, 2002], global hypomethylation in other types of cancer included the tumor-associated MAGE-A genes [Wischnewski *et al*. 2007]. Specific hypomethylation of the MAGE-A1 promoter in tumors was associated with inhibition of DNA methylation by transcription factor binding required to maintain an active promoter [De Smet *et al*. 2004]. Epigenetic activation of the MAGE gene family could be associated with activity similar to BORIS, an epigenetic reprogramming factor in the male germ line shown to activate MAGE-A subfamily gene expression in the testis [Hong *et al*. 2005; Loukinov *et al*. 2002]. The studies suggest that MAGE-11 and possibly other cancer–testis antigen genes are hormone regulated in normal reproductive tissues, and are subject to deregulated DNA hypomethylation that increases expression in advanced forms of cancer. Expression levels may be further enhanced through the activation of constitutive second messenger pathways since MAGE-11 levels increased in prostate cancer cells in response to cyclic AMP [Karpf *et al*. 2009].

The Xq28 chromosomal locus that contains the MAGE-11 gene harbors genes involved in over 40 diseases, with 17 diseases mapped to the region for which a gene has yet to be identified [Kolb-Kokocinski *et al*. 2006]. A hereditary form of prostate cancer has been linked to the Xq27-28 locus by gene linkage analysis [Brown *et al*. 2004; Xu *et al*. 1998], which raises the possibility that changes in MAGE-11 expression may be associated with hereditary prostate cancer [Stephan *et al*. 2002]. Cancer–testis antigens have also been identified in estrogen receptor-negative

forms of breast cancer that have increased expression of cancer–testis antigens that include the MAGE-A subfamily [Grigoriadis *et al.* 2009].

AR as a therapeutic target in castration-recurrent prostate cancer

AR has been the traditional prostate cancer drug target of the pharmaceutical industry and more recently of academic laboratories supported by the National Institutes of Health. Small molecule inhibitors can be effective agents because of their ability to diffuse freely into cells. The best-known AR small-molecule inhibitors are the antiandrogens used in the treatment of androgen-dependent prostate cancer as part of combined therapy with inhibition of gonadotrophin-releasing hormone. Antiandrogens competitively inhibit AR binding of androgen and AR transcriptional activity. Used in combined therapy, antiandrogens can prolong the period of remission [Akaza *et al.* 2009; Schmitt *et al.* 2001], but have not been effective in preventing castration-recurrent growth. The presence of testosterone and DHT at levels sufficient to activate AR [Titus *et al.* 2005b; Mohler *et al.* 2004] has escalated research on the intracrine synthesis of androgens in castration-recurrent prostate cancer cells [Knudsen and Penning, 2010; Locke *et al.* 2008; Montgomery *et al.* 2008; Stanbrough *et al.* 2006; Titus *et al.* 2005a, 2005b; Mohler *et al.* 2004] and renewed efforts to inhibit AR through inhibition of androgen biosynthesis [Reid *et al.* 2010; Attard *et al.* 2009].

The inability of antiandrogens to inhibit the growth of castration-recurrent prostate cancer even though AR is an essential transcription factor that drives tumor recurrence may have several causes. AR activated by local tissue androgen may not be effectively competed by antagonists that bind AR with lower affinity. AR binds testosterone and DHT with 100–500-fold higher affinity than it binds antiandrogens such as hydroxyflutamide, the active form of flutamide [Kemppainen *et al.* 1999]. If local tissue androgen production accounts for AR signaling and castration-recurrent growth, these lower affinity AR antagonists may not compete sufficiently with the more readily available higher affinity androgens. However, ligand-binding affinity alone does not differentiate agonists from antagonists. Some anabolic steroids bind AR with only moderate affinity but are potent androgens *in vivo*, in part because of their ability to induce the AR N/C interaction, whereas traditional AR antagonists inhibit the AR N/C interaction. Thus, to compete for local androgen production, higher-affinity AR antagonists are needed that inhibit the AR N/C interaction and AR signaling to block castration-recurrent prostate cancer growth.

On the other hand, numerous studies suggest that AR activation can be enhanced through alternative pathways independent of androgen binding [Ponguta *et al.* 2008; Culig and Bartsch, 2006; Culig *et al.* 2005] in which case antiandrogen or androgen deprivation therapy may be ineffective. Alternative AR transactivation pathways could provide potential new targets for small molecule inhibitors. Those currently being targeted include inhibition of AR binding to DNA and inhibition of AR interaction with coregulatory proteins.

The approach of small-molecule inhibitors that block ERα binding to DNA was recently developed as a potential therapy for breast cancer. First- and second-generation small-molecule inhibitors such as theophylline-8-[(benzylthio)-methyl]-(7CI,8CI) act outside the ERα ligand-binding pocket and inhibit ERα DNA binding and transcription and the estrogen-dependent growth of tamoxifen-resistant ERα positive MCF-7 breast cancer cells, with no effect on estrogen-independent cell growth [Mao *et al.* 2008]. A potential limitation of this approach is that transcriptional effects may occur independent of direct receptor binding to DNA [Carroll *et al.* 2006]. On the other hand, the functional effects of AR require binding to DNA based on complete androgen resistance that results from naturally occurring AR DNA binding mutations that cause the androgen insensitivity syndrome [Brown, 1995; Quigley *et al.* 1995]. Thus, a small-molecule inhibitor that targets AR binding to DNA may be a promising approach for new drug development.

MAGE-11 as a therapeutic target in castration-recurrent prostate cancer

Nuclear receptor interactions with coregulators is another treatment target in cancer [O'Malley, 2009]. Targeting protein–protein interactions with small-molecule inhibitors is a challenging but promising approach supported by recent reports. The proteasome inhibitor bortezomid (Velcade) was developed for the treatment of multiple myeloma [Arkin and Wells, 2004]. A high throughput screen using fluorescence

polarization that measured binding of c-Jun N-terminal kinase 1 (JNK1) interaction to its binding partner, JNK interacting protein 1 (JIP1), identified two classes of small-molecule inhibitors that allosterically block the JNK/JIP interaction site [Chen *et al*. 2009]. A small-molecule inhibitor class known as Nutlins inhibit mouse double minute 2 (mdm2) degradation of p53 [Graat *et al*. 2007]. Other less-specific targets for small-molecule inhibitors of AR action in prostate cancer include the inhibition of interactions with heat shock proteins [Hieronymus *et al*. 2006; Solit *et al*. 2003], histone deacetylases [Dobosy *et al*. 2007] and HER2-neu kinase [Mellinghoff *et al*. 2004].

Small-molecule inhibitors that target AR interactions with p160 coactivators or MAGE-11 may be a strategy to inhibit castration-recurrent prostate cancer growth [Karpf *et al*. 2009; Gregory *et al*. 2001]. Because MAGE-11 levels are low in most normal cells and increase during prostate cancer progression to recurrent growth, inhibition of the AR–MAGE-11 interaction may have minimal side effects. The potential effectiveness of small chemical inhibitors is enhanced by a well-defined interaction surface. The interaction between the carboxyl-terminal MAGE-11 F-box and AR NH_2-terminal F*XX*LF motif, where both are predicted to form an α-helical structure, may be amenable to inhibition by drug-like compounds [Askew *et al*. 2009; Bai *et al*. 2005; Arkin and Wells, 2004].

An alternative approach to inhibit AR signaling in castration-recurrent prostate cancer is to target MAGE-11 using immunotherapy. Cancer–testis antigens are immunogenic in cancer cells, and tumor-specific antigens can bring about antibody-directed tumor rejection. This and the largely restricted expression of cancer–testis antigens in cancer has led to the development of cancer vaccines [Scanlan *et al*. 2004, 2002]. Cancer–testis antigens are cleaved in cancer cells and presented on the cell surface in association with the human leukocyte antigen (HLA) class I (or major histocompatibility) complex where they elicit a cytotoxic T-cell and humoral response as shown in melanoma [Kalejs and Erenpreisa, 2005; Gaugler *et al*. 1994]. MAGE-A1 was the first tumor-specific antigen shown to be recognized by specific cytotoxic T cells [Hérin *et al*. 1987]. The vaccination of patients with MAGE-A peptides resulted in tumor regression [Mischo *et al*. 2006; Marchand *et al*. 2003; Sadanaga *et al*. 2001; Thurner *et al*. 1999]. NY-ESO-1 of the MAGE gene family is considered one of the most immunogenic cancer–testis antigens and has been the focus of humoral immune approaches in clinical trials for different types of cancer expressing MAGE-A and NY-ESO-1 [Atanackovic *et al*. 2008; Bender *et al*. 2007; Odunsi *et al*. 2007; Scanlan *et al*. 2004, 2002]. A similar immune therapy approach targeting MAGE-11 may provide an effective prostate cancer vaccine. However, it remains to be established whether MAGE-11 peptides are presented on the prostate cancer cell surface in association with the HLA class I complex and induce a cytotoxic T-cell immune response. It may be that cancer–testis antigen-directed tumor vaccines combined with epigenetic modulatory drugs that inhibit DNA methyltransferases and histone deacetylases [Karpf, 2006] will provide new approaches for the treatment of advanced prostate cancer.

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Conflict of interest statement

The author declares that there is no conflict of interest.

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