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Prostate Specific Antigen Gene Regulation by Androgen Receptor

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Abstract Prostate specific antigen (PSA) is a serine protease that is synthesized by both normal and malignant epithelial cells of the human prostate. PSA expressed by malignant cells, however, are released into the serum at an increased level, which can be detected to diagnose and monitor prostate cancer. Moreover, increases in serum PSA following local and systemic treatments are highly correlated with tumor recurrence and progression, and this association has further established PSA as a clinically important biomarker. The expression of PSA is mainly induced by androgens and regulated by the androgen receptor (AR) at the transcriptional level. Extensive research on the regulation of PSA gene expression has provided significant information about the function of AR, which is a crucial transcription factor involved in all phases of prostate cancer. Still, the molecular mechanism(s) by which the transcription of the PSA gene escapes regulation in advanced prostate cancer has yet to be clearly defined. Accumulating evidence suggests that a number of processes including androgen-independent activation of AR are involved. Lacking an effective treatment, advanced prostate cancer is almost invariably fatal, which highlights the importance of elucidating mechanisms of tumor progression. Insights into AR activity at the PSA gene could be extended to transcriptional regulation of other AR target genes, which may be crucial in discerning prostate cancer progression. Ultimately, our improved understanding of AR-regulated PSA expression could aid in developing viable therapies in treating and/or preventing advanced prostate cancer. J. Cell. Biochem. 93: 233–241, 2004.

Key words: PSA; prostate cancer; androgen receptor; transcription; gene regulation

Androgen action, mediated by the androgen receptor (AR), is essential for normal growth, differentiation, and maintenance of the prostate epithelium. The majority of prostate cancers are also androgen-dependent and respond initially to androgen ablation therapy, which is the only effective systemic treatment currently available [Denmeade and Isaacs, 2002]. Despite an initial favorable response, androgen ablation is essentially palliative, and disease progression eventually ensues [Kozlowski et al., 1991]. Prostate cancers that recur after androgen ablation are often responsive to subsequent alternative hormone manipulations that depend on a functional AR. However, tumors surviving multiple ablation therapies eventually progress to androgen-independence, at which point the tumors are far more aggressive and are invariably fatal. Although these hormone-refractory prostate cancers are operationally defined as being androgen-independent, they are thought to still depend on AR signaling for growth and continue to express high levels of AR and AR-regulated genes, such as prostate specific antigen (PSA). The mechanisms of AR activation in these clinically androgen-independent tumors are unclear and may represent critical processes in disease progression. Nevertheless, there are a number of proposed pathways that cancer cells may utilize to stimulate AR-mediated transcription under low androgen condition, and these include AR gene mutation and amplification, co-regulator alterations, and

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androgen-independent activation of the AR [Weber and Gioeli, 2004]. Although these mechanisms may operate simultaneously, emerging evidence suggests that the androgen-independent activation of the AR through cross-talk with various signal transduction pathways is a major contributor to this process. The key to understanding the molecular progression to androgen-independent prostate cancer may be to delineate the mechanisms involved in activation of AR-regulated genes such as PSA.

ANDROGEN RECEPTOR STRUCTURE AND MECHANISM OF ACTION

Substantial evidence amassed over the past 30 years since its identification indicates that AR is one of the putative susceptibility genes for prostate cancer. The AR gene encodes the transcription factor that regulates the expression of androgen-responsive genes involved in prostate epithelial cell division and differentiation. Moreover, both normal and malignant prostate epithelial cells are dependent on the AR signaling axis for growth, and AR expression is maintained in all forms of prostatic tumors [Buchanan et al., 2001; Feldman and Feldman, 2001]. The AR gene is located on the long arm of the X chromosome (Xq11-12) and comprises eight exons that encode an mRNA of 11 kb. The mRNA has a 2.8 kb open reading frame, a 1.1 kb 5′ untranslated region (UTR), and a 6 kb 3′ UTR. AR protein has four domains: an N-terminal domain (TAD) involved principally in transcriptional activation, a DNA-binding domain (DBD) that is required for interaction with specific gene sequences, a so-called hinge region, and a C-terminal ligand-binding domain (LBD) that binds androgens.

Testosterone is the main circulating androgen in males that is secreted primarily by testes and circulates in the blood, where it is bound to sex hormone binding globulin (SHBG). Upon entering prostate cells, 90% of testosterone is converted to the more active hormone, dihydrotestosterone (DHT), by the enzyme 5 alpha-reductase [Feldman and Feldman, 2001]. Similar to other nuclear receptors, the AR is bound to a multi-protein (including heat shock proteins) chaperone complex in its inactive state. Androgen binding induces a conformational change in the AR that leads to dissociation from the chaperone complex and phosphorylation of the receptor. This conformation change also facilitates dimerization of the AR, which then binds to the major groove of specific DNA sequences called androgen response elements (AREs) in the 5′ regulatory regions of target genes.

Subsequently, the ARE-bound AR homodimer recruits coactivators, such as the p160 and p300/CBP, which bridge interactions with the general transcription machinery and modify histones, thus activating gene expression [Rosenfeld and Glass, 2001]. Although many AR target genes have been identified to date, none has been as extensively studied as PSA.

PSA STRUCTURE AND FUNCTION

The PSA gene is a member of a family of genes encoding kallikrein-like serine proteases. A total of 15 tissue kallikrein genes have been identified through sequencing of the human genome, and they are clustered in the kallikrein locus at chromosome 19q13.3-13.4 [Diamandis and Yousef, 2001]. All of the kallikrein genes encode five exons of similar size and have significant homology with each other (40–80%). Many of these genes, such as *KLK2*, *KLK3* (PSA), and *KLK4*, are regulated by steroid hormones.

The PSA gene encodes a 33 kDa glycoprotein, which consists of a single polypeptide chain of 240 amino acids. In the normal prostate, PSA is secreted into the glandular ducts where it functions to degrade high molecular weight proteins synthesized in the seminal vesicles to inhibit coagulation of the semen [Balk et al., 2003]. PSA only enters the serum through leakage into the extracellular fluid of the normal prostate. However, during tumorigenesis, serum PSA levels are elevated due to the loss of the normal glandular architecture. As a result of being complexed to protease inhibitors such as alpha 1-antichymotrypsin, PSA in the circulation is usually inactive [Stenman et al., 1991]. The proteolytic activity of PSA may go beyond its normal function in prostate tumors and could possibly contribute to cancer progression. PSA has been shown to reduce the affinity of insulin-like growth factor binding protein-3 (IGFBP-3) for insulin-like growth factor-1 (IGF-1) through the proteolysis of IGFBP-3, which results in increased availability of mitogenic IGF-1 [Cohen et al., 1994]. Furthermore, the elevated levels of IGF-1 increased proliferation of cultured prostate epithelial cells. Also, PSA itself may increase the growth of primary tissue

cultures of prostatic epithelial cells. However, other in vitro experiments have failed to demonstrate a correlation between PSA function and tumor promoting activities [Balk et al., 2003]. In addition, human prostate cancer cell lines that do not express PSA, such as PC-3 and DU-145, are more tumorigenic and invasive than the LNCaP cell line, which produces PSA. Such conflicting results make it difficult to establish whether PSA has an active biological role in promoting the growth or progression of prostate cancer, and the significance of these mechanisms remains to be determined.

ANDROGEN REGULATED EXPRESSION OF PSA

The expression of PSA is primarily activated by androgens and regulated by the AR at the transcriptional level. AR regulated expression of PSA is mediated through AREs in the proximal promoter (−600 to +12) and the 5′ upstream enhancer (−3875 to −4325) of the PSA gene (Fig. 1). The proximal promoter contains a TATA box (−28/−23), ARE I (−170/−156), ARE II—also known as androgen response region (ARR;−395/−376), and SP-1 (−43/−54, −84/−93, −113/−123) [Sadar et al., 1999]. ARE I is a high affinity AR binding sequence (AGAACAgcaAGTGCT), which is closely related to the ARE consensus sequence (GGTACAnnnTGTTCT) [Riegman et al., 1991]. In contrast, ARR is a weak nonconsensus ARE sequence (GGATCAgggAGTCTC), which may cooperate with other AREs to increase the transcriptional potential of the promoter [Cleutjens et al., 1996]. The upstream enhancer was shown to be required for high androgen-stimulated PSA expression and was found to contain a single strong consensus ARE (ARE III; GGAACAtatTGTATC) [Cleutjens et al., 1996] and multiple additional weak nonconsensus AREs, which may also contribute to high androgen activity [Huang et al., 1999]. Another regulatory site present at the enhancer is the putative cAMP response element (CRE; TGACGTCA) located at −3196/−3189 [Sadar et al., 1999]. Although cAMP strongly induces the expression of the PSA gene by an AR-dependent pathway, it is not known whether transcription factors that are responsive to cAMP, such as cAMP responsive element binding protein (CREB) and activating transcription factor (ATF), regulate PSA gene expression by binding to this site.

Activation of PSA gene transcription involves many different proteins that are recruited directly (primary coactivators) and indirectly (secondary coactivators and general transcription machinery) by the AR. Primary coactivators include three related genes that encode the p160 coactivators, referred to as SRC-1, GRIP-1/TIF2, and pCIP/ACTR/RAC/AIB-1/TRAM-1, as well as Swi/Snf and TRAP/DRIP [Rosenfeld and Glass, 2001]. In addition, a number of AR coactivators have been identified that interact directly with AR, including a series of AR associated proteins (ARAs), gelsolin, and β-catenin [Rahman et al., 2004]. Also, it was shown that the tumor suppressor, BRCA1, can enhance AR-dependent transactivation by directly binding to AR and GRIP-1, which suggests an interesting interplay between a tumor suppressor and AR [Park et al., 2000]. Thus, the primary coactivators generally function by stabilizing the receptor complex and recruiting other coactivators. On the other hand, secondary coactivators, such as CREB binding protein (CBP)/p300 and coactivator-associated arginine methyltransferase 1(CARM1), promote transcription by binding to the p160 coactivators and covalently modifying the local histones,

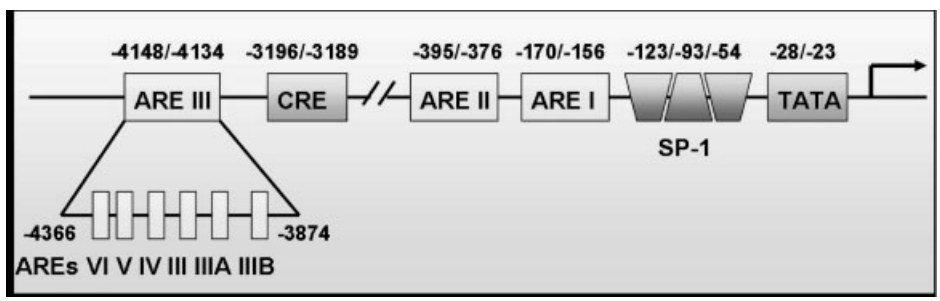

Fig. 1. The proximal promoter and upstream enhancer comprise the PSA 5′ regulatory regions that are required for high androgen-stimulated expression.

which allow access to the basal transcriptional machinery [Rosenfeld and Glass, 2001]. These coactivators may also stabilize the preinitiation complex by interacting directly with the components of the basal transcriptional machinery (Fig. 2). Consistent with this model, it was recently reported that androgen induces robust recruitment of AR, p160 coactivators, and CBP/p300 specifically to the PSA enhancer [Louie et al., 2003]. Furthermore, RNA polymerase II (pol II) was shown to be recruited to the enhancer independent of the proximal promoter, which taken together, support a model in which AR and associated coactivators mediate transcription initiation by serving as a staging platform for Pol II recruitment.

CHROMATIN MODIFICATION AT THE PSA GENE

The molecular processes that lead to activation or repression of transcription are emerging as the foundation for gene regulation. There has been a rapid accumulation of compelling evidence that points toward chromatin modification, a process that alters the transcription potential of nucleosomes, as a key determinant in gene regulation. It is becoming apparent that accessibility and activation of genes are largely dependent on diverse post-translational modifications of histone amino-termini. These modifications include acetylation, phosphorylation, and methylation which covalently add acetyl-, phospho-, or methyl-groups to specific residues of the N-terminal tail of core histones [Zhang and Reinberg, 2001]. The best characterized of the modifications is the acetylation of lysines on histones H3 and H4, which is correlated to transcriptional activation. Acetylation, catalyzed by histone acetyltransferases (HAT) such as p300/CBP, is reversed by the function of histone deacetylases (HDAC), which mediate transcriptional repression [Johnstone, 2002]. Other covalent modifications including phosphorylation of histone H3 at serine 10 and methylation of histones H3 and H4 are emerging as important factors that determine transcriptional status. Furthermore, the complex interactions between the different histone tail modifications have led to the proposal of 'histone code hypothesis,' which suggests that specific histone modifications affect and interact with other histone modifications and serve as marks for the recruitment of associated factors that regulate chromatin functions [Strahl and Allis, 2000].

While most of the studies on chromatin modifications have been performed on lower organisms such as *Saccharomyces cerevisiae* and *Drosophila melaganoster*, only recently chromatin modifications at the PSA gene in human were investigated. It was shown that histone H3 acetylation followed AR occupancy at the PSA promoter and enhancer, which is

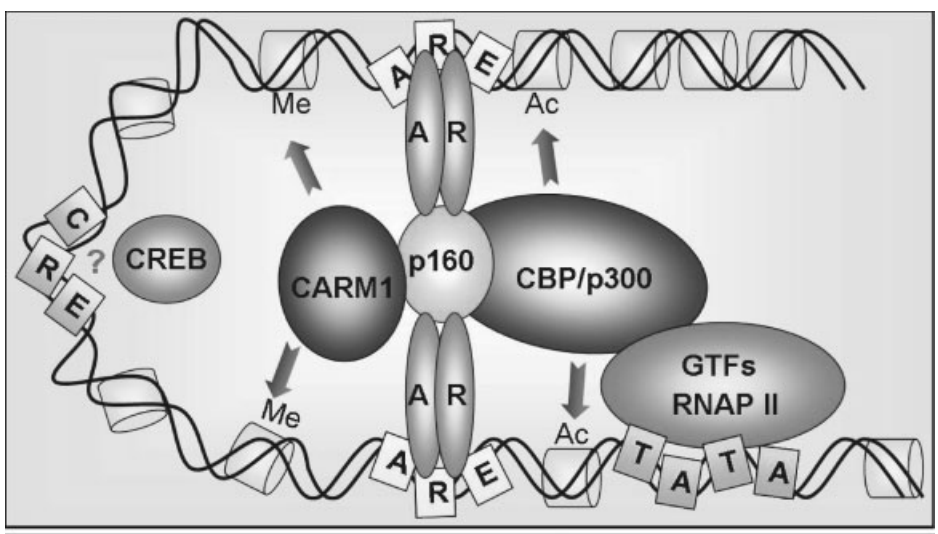

Fig. 2. Activation of PSA gene transcription involves AR-mediated recruitment of coactivators, general transcription factors (GTFs), and RNA polymerase II (RNAP II). The PSA promoter and enhancer may cooperate in proximity to activate transcription by bridged AR/coactivators/RNA polymerase II complexes.

consistent with p300/CBP recruitment to those regions by AR/p160 complex [Jia et al., 2003; Louie et al., 2003]. Also, AR-mediated transcription was accompanied by rapid decreases in di- and tri-methylated H3 at lysine 4 (K4) at both 5′ regulatory regions [Kim et al., 2003], which is contrary to the data in *Saccharomyces cerevisiae*, where tri-methylated H3-K4 is exclusively associated with active chromatin [Santos-Rosa et al., 2002]. The results may suggest a novel role for methylated H3-K4 in transcriptional regulation, possibly pointing to a yet to be discovered histone demethylase. An alternative hypothesis may be that methylated H3-K4 acts as a binding site for a protein associated with active transcription (possibly a coactivator), and thus the decrease in methylated H3-K4 observed with transcriptional activation by androgen is actually due to antibody epitope masking. Still another hypothesis is that the adjacent threonine 3 (H3-T3) could be phosphorylated, which may sterically hinder the methylation at H3-K4 (personal communication, C.D. Allis and J. Rice). Thus, the functional consequences of specific histone modifications at the PSA gene still remain to be determined. However, it is clear that our understanding of the histone modifications would provide important clues to the transcriptional regulation at the PSA gene.

PSA POLYMORHISMS AND PROSTATE CANCER RISK

The inevitable failure of androgen ablation therapy has led to strategies of developing methods to aggressively screen for cancers that are still confined locally to the prostate and so are potentially curable by specific treatment. Consequently, the correlation that exists between PSA expression level and prostate cancer development has established PSA as the most widely used biomarker for detecting and monitoring prostate cancer. However, the role of PSA in screening and staging of prostate cancer remains limited because of the broad and overlapping ranges in serum PSA levels that exist in many patients with either localized or advanced disease. Any condition that increases the volume of the prostate or disrupts the prostatic architecture, including benign prostatic hyperplasia and prostatitis, can elevate serum PSA levels [Balk et al., 2003]. Furthermore, factors such as age and race also have been shown to be associated with serum PSA levels, possibly due to differences in prostate volume.

Accruing evidence suggests that genetic variation in the promoter of the PSA gene may also contribute to individual variation in serum PSA levels. For example, serum PSA levels in healthy men are associated with a G/A single nucleotide polymorphism (SNP) at ARE I (at position −158) and/or the number of CAG repeats in exon 1 of the AR gene [Xue et al., 2001]. It was found that PSA levels are higher among healthy men with the AA genotype (at ARE I) compared to men with the AG or GG genotypes. The same SNP was reported to be associated with prostate cancer risk, with the GG genotype at significantly increased risk for advanced cancer [Xue et al., 2000]. Furthermore, shorter AR exon CAG repeats had increasing PSA effect on those genotypes of both healthy men and prostate cancer patients, suggesting a probable gene–gene interaction in the etiology of prostate cancer. However, other studies, involving separate groups of men, have shown that either the PSA AA genotype (as opposed to GG) was associated with increased cancer risk or not associated with prostate cancer at all [Medeiros et al., 2002; Xu et al., 2002]. These divergent results may be due to the different populations that exhibit disparate levels of genetic variation, environmental factors, and gene–environment interactions. Alternatively, a possible explanation comes from a study, which reported that additional SNPs present at the PSA enhancer (G/A at −4643, C/T at −5412, and T/G at −5429) are associated with serum PSA levels in healthy men, suggesting that there may be a linkage disequilibrium of the PSA promoter polymorphism with those at the enhancer region [Cramer et al., 2003]. Still, further studies are needed to clarify the potential association between genetic variation and prostate cancer risk.

ANDROGEN-INDEPENDENT INDUCTION OF PSA

It has been hypothesized that continued signaling of the AR in a castrate hormone environment could result from overexpression of the receptor, gain-of-function AR gene somatic mutations, coactivator overexpression, and ligand-independent activation of the AR [Buchanan et al., 2001; Feldman and Feldman,

2001; Balk, 2002]. As a result, the tumor cells may continue to proliferate and avert apoptosis by exploiting other available steroid hormones and growth factors in low androgen conditions.

Prostate tumors could survive and proliferate in the altered hormonal environment possibly by interactions between growth factor- and cytokine-activated pathways and AR signaling. Indeed, recent evidence indicates that a number of growth factors and cytokines such as insulin-like growth factor 1 (IGF-1), keratinocyte growth factor (KGF), epidermal growth factor (EGF), forskolin (FSK), and interleukin 6 (IL-6) have been shown to stimulate AR signaling in the absence of androgen [Culig et al., 1994; Nazareth and Weigel, 1996; Ueda et al., 2002]. Various signal transduction pathways and mechanisms of activation seem to be involved in the function of these alternative ligands (Fig. 3). Both EGF and FSK increase AR phosphorylation at serine 650, which could stimulate AR transactivation [Gioeli et al., 2002]. These signaling molecules may also act through divergent mechanisms. EGF signaling through mitogen activated protein kinase (MAPK) pathway was shown to increase TIF2/GRIP1 coactivation of AR transactivation in recurrent prostate cancer [Gregory et al., 2004], while FSK is able to induce PSA gene expression in LNCaP cells by activating the amino-terminus of the AR and increasing protein–DNA complex formation between AR and PSA-ARE [Sadar et al., 1999]. Another growth factor, IGF-1, has been implicated in prostate cancer cell proliferation as mentioned above. Consistent with a positive correlation between elevated serum IGF-1 levels and increased prostate cancer risk [Chan et al., 1998], IGF-1 enhanced

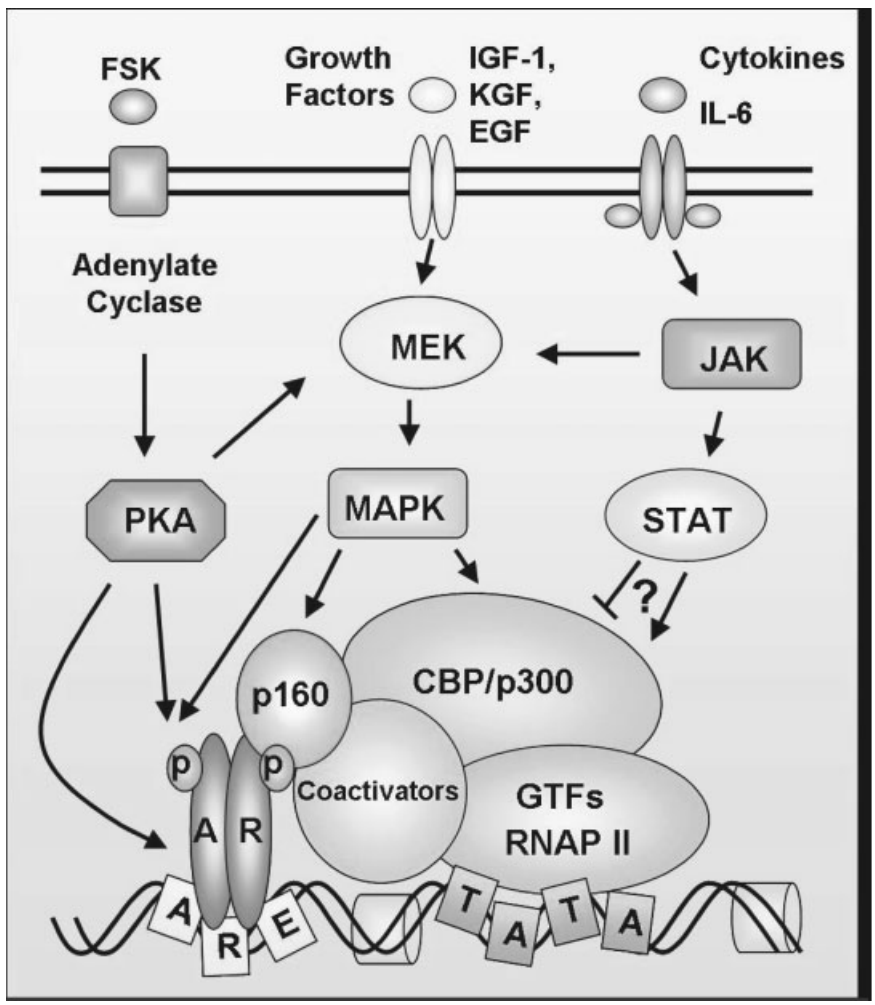

Fig. 3. Androgen-independent activation of PSA gene transcription via various signal transduction pathways.

the magnitude of the AR response in the presence of low levels of androgen, suggesting that the cross-talk between AR and growth factor signaling pathways may sensitize AR to suboptimal stimulation by low levels of androgens [Orio et al., 2002].

Besides growth factors, cytokines, such as IL-6, are involved in prostate cancer progression. IL-6 levels in patients with therapy resistant prostate tumors were elevated [Twillie et al., 1995], and moreover, IL-6 elevation over 7 pg/ml was found to be associated with poor prognosis in multivariate analysis [Nakashima et al., 2000]. Spurred by these clinical findings, a number of groups reported that IL-6 enhances AR transactivation. IL-6 was shown to enhance AR transactivation via either signal transducers and activators of transcription-3 (STAT3) or MAPK pathways [Chen et al., 2000; Ueda et al., 2002]. It was further proposed that CBP/p300 mediates this IL-6 effect [Debes et al., 2002]. Moreover, Chang et al. found that whereas IL-6 enhanced AR transactivation activity via either the STAT3 or MAPK pathways, it suppressed AR transactivation activity via the Akt pathway [Yang et al., 2003]. Still, another study indicates that IL-6 can induce androgen responsiveness in prostate cancer cells through upregulation of AR expression [Lin et al., 2001]. However, recent findings indicate that IL-6 can also inhibit PSA expression and prostate cancer cell proliferation through inhibition of p300 recruitment to the PSA enhancer and promoter [Jia et al., 2003; Jia et al., 2004]. Furthermore, the inhibitory activity of IL-6 is, at least in part, mediated through the STAT3 pathway without MAPK involvement. These conflicting reports may be explained by a finding that long-term treatment of the prostate cancer cell line, LNCaP, with IL-6 abolishes its inhibitory growth response [Hobisch et al., 2001]. Thus, the stimulatory effects reported may be due to the transition of IL-6 from a paracrine growth inhibitor to an autocrine growth stimulator [Chung et al., 1999].

CONCLUSIONS

PSA has been widely investigated as a model gene for determining the mechanism by which AR-mediated transactivation occurs in normal and malignant prostate cells. It is clear, though, that many molecular aspects of AR-mediated expression of PSA remain to be elucidated in order to delineate the mechanisms of prostate cancer progression. Furthermore, the high level of PSA expression associated with tumor progression, taken together with its proteolytic activity, suggests the possibility that PSA may play a functional role in progression itself, by either direct or indirect stimulation of cancer cell growth or invasion. Continued expansion of our understanding of functions of AR and PSA will undoubtedly contribute to more effective therapies, as current therapies are usually beneficial in the earlier stages of cancer but are essentially palliative in more advanced or recurring androgen-insensitive tumors. Moreover, as the relationships between various cytokines, growth factors, and AR as well as the physiologic manifestations of androgen action are understood, the possible targets for pharmacologic intervention will be clarified. These imperative tasks represent an exciting challenge in prostate cancer research.

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REFERENCES

Balk S. 2002. Androgen receptor as a target in androgen-independent prostate cancer. Urology 60:132–138.

Balk SP, Ko YJ, Bubley GJ. 2003. Biology of prostate-specific antigen. J Clin Oncol 21:383–391.

Buchanan G, Irvine RA, Coetzee GA, Tilley WD. 2001. Contribution of the androgen receptor to prostate cancer predisposition and progression. Cancer Metastasis Rev 20:207–223.

Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M. 1998. Plasma insulin-like growth factor-I and prostate cancer risk: A prospective study. Science 279:563–566.

Chen T, Wang LH, Farrar WL. 2000. Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. Cancer Res 60:2132–2135.

Chung TD, Yu JJ, Spiotto MT, Bartkowski M, Simons JW. 1999. Characterization of the role of IL-6 in the progression of prostate cancer. Prostate 38:199–207.

Cleutjens KB, van Eekelen CC, van der Korput HA, Brinkmann AO, Trapman J. 1996. Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specific antigen promoter. J Biol Chem 271:6379–6388.

Cohen P, Peehl DM, Graves HC, Rosenfeld RG. 1994. Biological effects of prostate specific antigen as an insulin-like growth factor binding protein-3 protease. J Endocrinol 142:407–415.

Cramer SD, Chang BL, Rao A, Hawkins GA, Zheng SL, Wade WN, Cooke RT, Thomas LN, Bleecker ER, Catalona WJ, Sterling DA, Meyers DA, Ohar J, Xu J. 2003. Association between genetic polymorphisms in the prostate-specific antigen gene promoter and serum prostate-specific antigen levels. J Natl Cancer Inst 95: 1044–1053.

Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G, Klocker H. 1994. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res 54:5474–5478.

Debes JD, Schmidt LJ, Huang H, Tindall DJ. 2002. p300 mediates androgen-independent transactivation of the androgen receptor by interleukin 6. Cancer Res 62:5632–5636.

Denmeade SR, Isaacs JT. 2002. A history of prostate cancer treatment. Nat Rev Cancer 2:389–396.

Diamandis EP, Yousef GM. 2001. Human tissue kallikrein gene family: A rich source of novel disease biomarkers. Expert Rev Mol Diagn 1:182–190.

Feldman BJ, Feldman D. 2001. The development of androgen-independent prostate cancer. Nat Rev Cancer 1:34–45.

Gioeli D, Ficarro SB, Kwiek JJ, Aaronson D, Hancock M, Catling AD, White FM, Christian RE, Settlage RE, Shabanowitz J, Hunt DF, Weber MJ. 2002. Androgen receptor phosphorylation. Regulation and identification of the phosphorylation sites. J Biol Chem 277:29304–29314.

Gregory CW, Fei X, Ponguta LA, He B, Bill HM, French FS, Wilson EM. 2004. Epidermal growth factor increases coactivation of the androgen receptor in recurrent prostate cancer. J Biol Chem 279:7119–7130.

Hobisch A, Ramoner R, Fuchs D, Godoy-Tundidor S, Bartsch G, Klocker H, Culig Z. 2001. Prostate cancer cells (LNCaP) generated after long-term interleukin 6 (IL-6) treatment express IL-6 and acquire an IL-6 partially resistant phenotype. Clin Cancer Res 7:2941–2948.

Huang W, Shostak Y, Tarr P, Sawyers C, Carey M. 1999. Cooperative assembly of androgen receptor into a nucleoprotein complex that regulates the prostate-specific antigen enhancer. J Biol Chem 274:25756–25768.

Jia L, Kim J, Shen H, Clark PE, Tilley WD, Coetzee GA. 2003. Androgen receptor activity at the prostate specific antigen locus: Steroidal and non-steroidal mechanisms. Mol Cancer Res 1:385–392.

Jia L, Choong CS, Ricciardelli C, Kim J, Tilley WD, Coetzee GA. 2004. Androgen receptor signaling: Mechanism of interleukin-6 inhibition. Cancer Res 64:2619–2626.

Johnstone RW. 2002. Histone-deacetylase inhibitors: Novel drugs for the treatment of cancer. Nat Rev Drug Discov 1:287–299.

Kim J, Jia L, Tilley WD, Coetzee GA. 2003. Dynamic methylation of histone H3 at lysine 4 in transcriptional regulation by the androgen receptor. Nucleic Acids Res 31:6741–6747.

Kozlowski JM, Ellis WJ, Grayhack JT. 1991. Advanced prostatic carcinoma. Early versus late endocrine therapy. Urol Clin North Am 18:15–24.

Lin DL, Whitney MC, Yao Z, Keller ET. 2001. Interleukin-6 induces androgen responsiveness in prostate cancer cells through up-regulation of androgen receptor expression. Clin Cancer Res 7:1773–1781.

Louie MC, Yang HQ, Ma AH, Xu W, Zou JX, Kung HJ, Chen HW. 2003. Androgen-induced recruitment of RNA polymerase II to a nuclear receptor-p160 coactivator complex. Proc Natl Acad Sci USA 100:2226–2230.

Medeiros R, Morais A, Vasconcelos A, Costa S, Pinto D, Oliveira J, Carvalho R, Lopes C. 2002. Linkage between polymorphisms in the prostate specific antigen *ARE1* gene region, prostate cancer risk, and circulating tumor cells. Prostate 53:88–94.

Nakashima J, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H, Murai M. 2000. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. Clin Cancer Res 6:2702–2706.

Nazareth LV, Weigel NL. 1996. Activation of the human androgen receptor through a protein kinase A signaling pathway. J Biol Chem 271:19900–19907.

Orio F, Jr., Terouanne B, Georget V, Lumbroso S, Avances C, Siatka C, Sultan C. 2002. Potential action of IGF-1 and EGF on androgen receptor nuclear transfer and transactivation in normal and cancer human prostate cell lines. Mol Cell Endocrinol 198:105–114.

Park JJ, Irvine RA, Buchanan G, Koh SS, Park JM, Tilley WD, Stallcup MR, Press MF, Coetzee GA. 2000. Breast cancer susceptibility gene 1 (*BRCAI*) is a coactivator of the androgen receptor. Cancer Res 60:5946–5949.

Rahman M, Miyamoto H, Chang C. 2004. Androgen receptor coregulators in prostate cancer: Mechanisms and clinical implications. Clin Cancer Res 10:2208–2219.

Riegman PH, Vlietstra RJ, van der Korput JA, Brinkmann AO, Trapman J. 1991. The promoter of the prostate-specific antigen gene contains a functional androgen responsive element. Mol Endocrinol 5:1921–1930.

Rosenfeld MG, Glass CK. 2001. Coregulator codes of transcriptional regulation by nuclear receptors. J Biol Chem 276:36865–36868.

Sadar MD, Hussain M, Bruchovsky N. 1999. Prostate cancer: Molecular biology of early progression to androgen independence. Endocr Relat Cancer 6:487–502.

Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, Schreiber SL, Mellor J, Kouzarides T. 2002. Active genes are tri-methylated at K4 of histone H3. Nature 419:407–411.

Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. 1991. A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: Assay of the complex improves clinical sensitivity for cancer. Cancer Res 51: 222–226.

Strahl BD, Allis CD. 2000. The language of covalent histone modifications. Nature 403:41–45.

Twillie DA, Eisenberger MA, Carducci MA, Hseih WS, Kim WY, Simons JW. 1995. Interleukin-6: A candidate mediator of human prostate cancer morbidity. Urology 45:542–549.

Ueda T, Mawji NR, Bruchovsky N, Sadar MD. 2002. Ligand-independent activation of the androgen receptor by IL-6 and the role of the coactivator SRC-1 in prostate cancer cells. J Biol Chem 277:38087–38094.

Weber MJ, Gioeli D. 2004. Ras signaling in prostate cancer progression. J Cell Biochem 91:13–25.

Xu J, Meyers DA, Sterling DA, Zheng SL, Catalona WJ, Cramer SD, Bleecker ER, Ohar J. 2002. Association studies of serum prostate-specific antigen levels and the genetic polymorphisms at the androgen receptor and prostate-specific antigen genes. Cancer Epidemiol Biomarkers Prev 11:664–669.

Xue W, Irvine RA, Yu MC, Ross RK, Coetzee GA, Ingles SA. 2000. Susceptibility to prostate cancer: Interaction between genotypes at the androgen receptor and prostate-specific antigen loci. Cancer Res 60:839–841.

Xue WM, Coetzee GA, Ross RK, Irvine R, Kolonel L, Henderson BE, Ingles SA. 2001. Genetic determinants of serum prostate-specific antigen levels in healthy men from a multiethnic cohort. Cancer Epidemiol Biomarkers Prev 10:575–579.

Yang L, Wang L, Lin HK, Kan PY, Xie S, Tsai MY, Wang PH, Chen YT, Chang C. 2003. Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells. Biochem Biophys Res Commun 305:462–469.

Zhang Y, Reinberg D. 2001. Transcription regulation by histone methylation: Interplay between different covalent modifications of the core histone tails. Genes Dev 15:2343–2360.