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THE ANDROGEN RECEPTOR: A MEDIATOR OF DIVERSE RESPONSES

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1. ABSTRACT

Androgens mediate a number of diverse responses through the androgen receptor, a 110 kD ligand-activated nuclear receptor. Androgen receptor expression, which is found in a variety of tissues, changes throughout development, aging, and malignant transformation. The androgen receptor can be activated by two ligands, testosterone and dihydrotestosterone, which bind to the androgen receptor with different affinities. This difference in binding affinity results in different levels of activation of the androgen receptor by the two ligands. The androgen receptor acts as a transcriptional modifier of a variety of genes by binding to an androgen response element. The ability to confer androgen specific actions by the androgen response element may depend on other cell-specific transcription factors and *cis*-acting DNA elements in close proximity to it.

Testosterone and dihydrotestosterone appear to act upon an identical nuclear receptor. However, in certain instances, they mediate different physiologic responses. For example, dihydrotestosterone, but not testosterone, is capable of mediating full sexual development of the male external genitalia. In some cases, the androgen receptor may induce opposite physiologic responses in similar tissue types depending on their location. For example, in male pattern baldness, activated androgen receptors may suppress the growth of distinct hair follicle populations through initiating stromal-epithelial actions, whereas other hair follicles continue to proliferate. In other cases, altered androgen receptor activity due to its mutation or altered expression may lead to pathology such as recurrence of prostate cancer due to development of androgen independence allowing tumor cell proliferation under androgen deprivation.

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2. INTRODUCTION

Two important androgens, testosterone (T) and its metabolite dihydrotestosterone (DHT), affect a diversity of responses in a variety of tissues. They mediate their effects through a ligand-dependent nuclear transcription factor, the androgen receptor (AR). Cloning of the AR cDNA (1-4) has allowed for cellular and molecular characterization of many different aspects of androgen physiology and pathophysiology. However, in spite of the intensity of research activity in this area, many interesting questions remain regarding the basic functions of androgen and AR on gene regulation. In this review, we will provide a brief overview of the AR and its regulation and then explore several intriguing aspects of the cellular and molecular biology of androgens and their receptor.

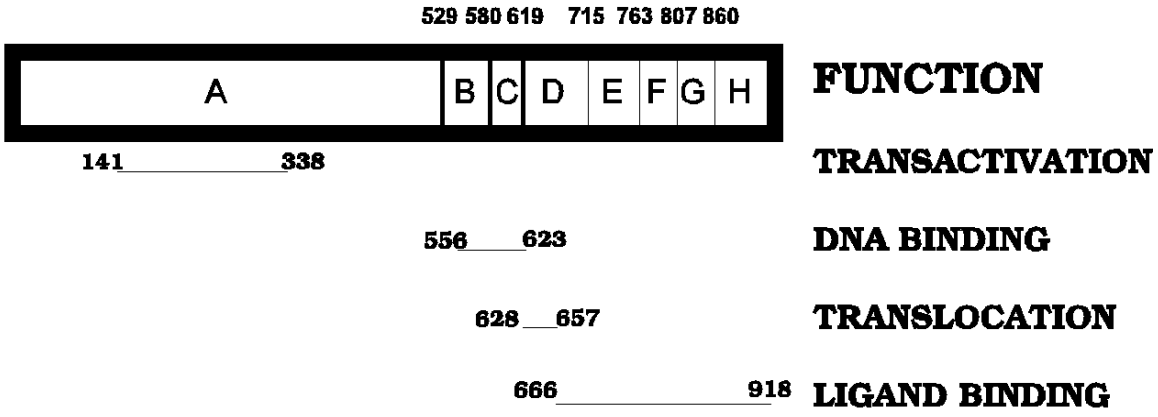

Figure 1. Schematic illustration of the functional domains of the human androgen receptor. The exons are labeled A-H. The numbers above the box indicate the amino acid number at the junction between the exon and the intron. The domains responsible for their specific function are also indicated by lines with the numbers of amino acid residues.

3. AN OVERVIEW OF THE ANDROGEN RECEPTOR.

3.1 Androgen Receptor Structure

The AR is a member of the steroid hormone receptor family. These receptors regulate gene transcription by interacting with a specific DNA sequence in a ligand-dependent manner. The AR is a 110 kD nuclear protein which consists of approximately 918 amino acid residues (1, 3, 5). Similar to other steroid hormone receptors, the AR consists of transactivation (6-10), DNA binding (11-16), nuclear localization (17-19), dimerization (20-22), and ligand binding domains (6, 23-27) (Fig. 1). Of these domains, the N-terminus is the most variable while the other regions are highly conserved. Within the N-terminus, the region of amino acids from 141 to 338 consist of polyglutamine and polyproline residues (3) which appear important in transcriptional activation (18, 28). The DNA-binding domain consists of 68 amino acids which fold into two zinc fingers capable of binding DNA. Four cysteine residues non-covalently bind the zinc ion within each finger. The remaining 295 amino acids form the C-terminus and encode the hinge region/nuclear localization signal and the ligand-binding domain.

3.2 Distribution of the androgen receptor

As demonstrated by a variety of methods, AR is present in most tissues. ^{3}H-androgen binding assays were the initial approach used to determine tissue distribution of AR. By injecting (^{3}H)-testosterone into rats followed by quantitation of (^{3}H)-androgen uptake in various tissues, Gustafsson and Pousette (29) were able to identify target organs of androgens. The development of anti-AR antibodies has complemented the autoradiographic methods of AR detection (30-33). Using anti-AR antibodies, Takeda *et al*. (32) evaluated AR distribution in various rat tissues. They were able to demonstrate that all male sexual organs in the rat showed a strong positive nuclear staining for AR, whereas several other tissues, including hepatic, renal, neuronal, muscular and female reproductive organs had weak, albeit positive, nuclear staining. In fact, the only tissue which did not demonstrate staining for AR was the spleen. The use of microwave-based antigen retrieval for AR (34) enhanced the immunohistochemical detection of AR in paraffin sections allowing for evaluation of archival tissue sections.

In addition to identification of AR protein, detection of AR mRNA in tissues has been accomplished by several methods. Cloning of human AR (hAR) and rat AR (rAR) cDNA (1, 35) allowed for development of probes which were used to detect in various tissues the AR mRNA by Northern blotting or by *in situ hybridization*. In addition to confirming previous immunohistochemical staining data, the analysis of AR mRNA by Northern blot resulted in identification of two isoforms in the A/B domain of AR mRNA in the vocal organ of *Xenopus* (36) and the brain of rodents (37). The sensitivity of detection of AR mRNA was greatly improved by competitive RT-PCR (38). Table 1. summarizes the relative expression (compared to prostate) of AR mRNA in various tissues of male and female Sprague-Dawley rats based on competitive RT-PCR (38). These results demonstrate agreement between mRNA levels and immunohistochemical staining intensity (39). One notable exception was the ability to demonstrate AR mRNA in the spleen using RT-PCR (38) as opposed to the inability to detect AR protein by immunohistochemical staining (32). This result is most likely attributable to the extreme sensitivity of RT-PCR as compared to immunohistochemistry.

3.3 Regulation of androgen receptor expression

AR expression is modified during fetal development, sexual development, aging, and

Table 1. Relative abundance of androgen receptor mRNA in various organs to the level in the prostate gland

Organ	Male	Female
Hypothalamus	42	217
Adrenal gland	141	186
Epididymis	115	ND
Thyroid gland	68	ND
Harderian gland	58	ND
Pituitary gland	56	9
Preputial gland	44	38
Quadriceps muscle	35	ND
Levator ani muscle	30	ND
Kidney	27	7
Coagulating gland	25	ND
Seminal vesicle	25	ND
Testis	20	ND
Liver	18	9
Submaxillary gland	17	ND
Bulbocavernosus muscle	16	ND
Vagina	ND	9
Heart	8	7
Ovary	ND	4
Uterus	ND	2

The relative abundance of androgen receptor (AR) mRNA in rat tissues (38). Total RNA from the indicated tissues was subjected to competitive reverse transcription-polymerase chain reaction for quantitation of AR mRNA levels. The data are reported as percentage of AR mRNA relative to the prostate AR mRNA levels (averaged from 5 male rats). The data represent mean values from 3-5 rats. ND = not determined.

malignant transformation. Regulation of AR levels may occur anywhere along the path from AR gene transcription to post-translational modification. A variety of factors, including androgens, have been implicated in modulating the AR protein and mRNA expression.

In the case of mouse fetal development, AR mRNA, based on *in situ* hybridization, was not found in the urogenital sinus at 13.5 days of gestation, whereas at 15.5 days of gestation both AR mRNA and protein levels were detectable (39).In the rat neonate, castration at 3 days of age did not result in altered AR expression in the rat prostate (40). In contrast, castration in the adult rat altered AR mRNA and protein levels (41-43). These findings suggest that one or more developmentally regulated factor(s) influence the AR expression. An age-dependent decline in hepatic rAR expression has been shown to be associated with the expression of an Age-dependent factor (ADF) which is ubiquitously expressed in tissues (44). ADF binds to a rAR fragment between -310 to -330. Rat hepatic ADF binding activity on the rAR promoter *in vitro* was shown to decrease with age, When the ADF binding site was mutated in a reporter construct, a decreased rAR promoter activity was observed.

As discussed below, in prostate cancer, development of an androgen independent state is associated with a heterogeneous AR expression. In addition to prostate cancer, the AR protein or mRNA has been detected in other forms of cancer such as hepatomas (45), germ-cell neoplasias (46), and ovarian tumors (47). These findings suggest that dedifferentiation may result in expression of factors which modulate the AR expression.

Several factors have been reported to modify the AR expression. These factors include androgens which were shown to decrease the AR mRNA expression in the rat ventral prostate (42, 48), a human androgen-responsive prostate carcinoma cell line (LNCaP) (42, 49), and a hepatoma cell line (HepG2) (48). However, these observations are controversial because up-regulation of AR by androgens has been demonstrated in the rat and mouse prostate (43), genital skin fibroblasts (50), rat penile smooth muscle (51), and male rat fat-pad adipose precursor cells (52). Some of these conflicting observations may be due to different methodologies used such as moderately sensitive Northern blots versus highly sensitive *in situ* hybridization. However, the presence of tissue-specific transcription factors may also account for the opposite effects androgens play on AR expression.

The regulation of the AR also takes place at the protein level. For example, in the androgen-dependent prostate cancer cell line, PC-82, although the AR mRNA level was stable AR protein was up-regulated by androgen (53). On the other hand, it was reported that the down-regulation of AR mRNA by androgen in the LNCaP line was associated with an increased AR protein expression (42, 54, 55). Mizokami *et al.* showed that the AR protein up-regulation by androgen resulted from enhanced stability of AR protein (55) providing a clue for these discrepant observations.

In addition to androgen, several other hormones and growth factors can regulate the AR expression. Follicle-stimulating hormone increases the level of mRNA of AR in the Sertoli cells (56). Growth hormone, prolactin (57) and epidermal growth factor (55, 58) increase the AR mRNA levels in prostatic cells. Interestingly, *trans*-retinoic acid down-regulated AR mRNA in T47D breast cancer cells but up-regulated the expression of AR mRNA in MDA-MB-453 breast cancer cells (59) demonstrating that the effect of the signal depends on the cell type.

In conclusion, AR expression can be modified by a variety of factors which appear to act in a tissue and cell specific fashion. Close examination

of the AR gene control elements may provide clues as to how this regulation is achieved.

3.4 The androgen receptor gene 5'-flanking region

Transcriptional control of the AR gene has been examined in a variety of conditions. The AR promoter lacks TATA and CCAAT boxes, but appears to have a GC box (a Sp1 binding site) which may play an important role in transcriptional initiation (60, 61). Additionally, it appears that there are two transcription initiation sites located 1127 base pairs (bp) and 1116 bp upstream of the translation initiation site (61, 62). A variety of putative *cis*-acting elements are present on the AR promoter (63). A cAMP response element (CRE) has been shown to stimulate AR transcription in both the human and murine AR (63-65). Though not reported for the human AR, the rAR promoter contains a NF-κB site which down-regulates transcription of the AR (66). In addition to the above studies, a *cis*-acting region between +57 and +575 of the 5'-untranslated region (5'-UTR) of the AR gene was identified which up-regulated an AR reporter gene's protein expression (62). However, this occurred without increasing the steady state mRNA levels suggesting that this region of the 5'-UTR functions by inducing translation (67).

4. HOW DO ANDROGENS ACHIEVE DISTINCT EFFECTS ON VARIOUS EPITHELIAL POPULATIONS?

Perhaps the most dramatic example of the difference of T and DHT actions are observed during male sexual development. It has long been recognized that T is required for the development of the internal accessory sexual organs, whereas DHT is required for the development of external sex organs. In humans, these events appear to be triggered by T expression which begins at the 8^{th} week of gestation (68). T, secreted by the Leydig cells, stimulates the Wolffian duct to differentiate into the epididymis, vas deferens, and seminal vesicles. The observation that unilateral orchiectomy results only in ipsilateral loss of Wolffian duct differentiation suggests that high concentrations of T are required for this event. The action of 5α-reductase on T results in the production of DHT which stimulates the differentiation of the urogenital sinus into the prostate, penis, and scrotum. The importance of DHT in this role is demonstrated in individuals with congenital 5α-reductase deficiency. Even though genotypically male (46, X, Y), these individuals cannot convert T to DHT and thus develop feminized external genitalia (69). However, because T is present during development, Wollfian duct differentiation proceeds normally resulting in formation of a pseudohermaphrodite. When coincident with puberty the level of T increases some of these individuals develop male characteristics such as increased skeletal muscle bulk, axillary and pubic hair growth, and maturation of the penis (70). This observation suggests that high levels of T may replace the loss of DHT. In agreement with this hypothesis are the following observations made by Imperato-McGinley *et al* (71): 1) flutamide, an androgen receptor blocker, administered to male rats *in utero* during the period of sexual differentiation completely inhibited the development prostate; and 2) administration of finasteride, a 5-α reductase inhibitor, resulted only in partial inhibition of development of prostate. Taken together, these results suggest that T, via the AR, can compensate for lack of DHT.

If as discussed below one subscribes to the idea that both T and DHT mediate their effects by interacting with a single nuclear receptor protein, then how can we account for the requirement of both T and DHT for sexual development? The answer may be found by examining the ability of these androgens to interact with the AR. Several investigators have demonstrated that T has approximately 3 times faster association and dissociation rates than DHT on both the rat (72) and human (73, 74) AR. In agreement with these binding kinetics, Zhou *et al.* have demonstrated that T is less effective at stabilizing AR than DHT (74). These observations suggest that DHT, by enhancing the stabilization of AR and its action, amplifies the T signal in those tissues which contain 5α-reductase. Perhaps in these tissues, the AR expression is not sufficient for T to mediate a physiologic response, but due to the ability of DHT to enhance AR activity, a response is observed.

In contrast to the inability of T to mediate various androgen-dependent events during sexual development, the biology of hair growth offers an example of the different effects that androgens exert on the proliferation of similar populations of epithelial cells. Specifically, T can stimulate facial hair growth, but causes the regression of scalp hair in aging individuals (75). Hair follicles are intimately associated with the mesenchymally-derived dermal papilla which is believed to provide an important influence on the follicular proliferation. Several lines of evidence support the role of androgens in controlling growth of hair follicles through modulation of the dermal papilla activity: 1) AR has been identified by both ligand binding assay (76) and by immunohistochemical staining in the dermal papilla (77); 2) dermal papillae from androgen-dependent hair follicles contain a greater number of AR than those in non-balding areas (78); 3) in primary dermal papilla cell lines from either androgen-dependent (i.e., beard) or -independent (i.e., non-balding scalp) hair follicles, only the beard dermal papilla-derived cells were able to synthesize DHT when T was added to the media (75); 4) the level of 5α-reductase varied between hair follicles from frontal and occipital sites (79); and 5) dermal papilla can produce extracellular matrix components and mitogenic factors (75). Taken together, these findings suggest that androgen, via the AR, can

indirectly mediate an effect on hair follicle proliferation through modulating dermal papilla activity. Though these data do not explain how T modulates cell proliferation in hair follicles, the differences in androgen metabolism and AR expression may, in part, account for the opposite proliferative responses observed in various epithelial tissues. The effect of androgens on cell proliferation in hair follicles may be regulated through regulation of expression of growth factors. Androgens can modulate expression of a variety of growth factors in the prostate stroma.

Mesenchymal-epithelial interactions are critical in the development of prostate tissue (reviewed in (80)). Prostate mesenchyme, when coincubated with Sertoli cells, synthesizes extracellular matrix in an androgen-independent manner; however, when exposed to androgen it produces a diffusible substance similar in action to P-Mod-S (81). P-Mod-S, a protein secreted by peritubular cells, can activate a variety of responses such as inhibin production and aromatase activity in Sertoli cells. Its effect on the prostate epithelia is currently unknown; however, it may also stimulate the activity similar to that observed in Sertoli cells thus accounting for one mechanism of mesenchymal-epithelial interaction within the prostate. Gleave et al. demonstrated that prostate fibroblasts secrete a diffusible substance which can stimulate the growth of LNCaP prostate carcinoma cells (82). Androgen deprivation mediated by castration resulted in expression of tenascin in the rat prostate (83).

5. WHY ARE THERE TWO ANDROGENS (TESTOSTERONE AND DIHYDRO TESTOSTERONE) ACTIVE ON ONE ANDROGEN RECEPTOR?

T and DHT mediate their effects by altering gene expression in target tissues via a single receptor, the AR. Despite the binding of T and DHT to a single receptor, the effect of these hormones on a single gene may be quite distinct. For example, differential regulatory effects of T and DHT on expression of several genes including Far-17a (84), and the cytokines interleukin-4 (IL-4), IL-5 and γ-interferon (γIFN) has been reported (85). The observation that both T and DHT can differentially regulate the expression of the androgen responsive genes has led to the controversial idea that more than one AR may exist. This hypothesis is supported by the following observations (reviewed in (86)) : (1) (^{3}H)T, when injected into rats, concentrates in the hypothalamic nuclei and 100x unlabeled DHT does not inhibit this localization; (2) hypothalamic localization is not observed after injection of (^{3}H)DHT; and (3) the ability of T, but not DHT to induce neuronal proliferation or male sexual behavior in castrated male rats. These data are consistent with two different possibilities. One possibility is that two different androgen receptors indeed exist. On the other hand, these data may be due to different metabolic interactions of the androgens on a single androgen receptor. As discussed above, T dissociates from the AR 3 times faster than DHT and is less effective in stabilizing the AR (74). This difference in the dissociation rate has been directly related to the androgens' ability to stimulate transcription of an androgen responsive gene (87). These observations could account for the differential effects of these androgens mediated by one receptor.

Perhaps the strongest evidence for the presence of one androgen receptor is derived from the observation that genotypic XY mice and rats with testicular feminization syndrome (Tfm) do not have a fully functional AR (87-90) and even though they express both T and DHT they develop into phenotypic females. This experiment of nature demonstrates that loss of one AR can result in loss of action of both androgens.

The presence of two androgens acting on one receptor may serve several possible functions. Cells which contain steroid 5α-reductase can convert T into DHT. Thus, the overall effect of this system may be to amplify the action of T via conversion to DHT within these cells, thus providing a mechanism of local regulation.

6. WHY ARE BOTH CONSENSUS AND NON-CONSENSUS PRESENT?

As mentioned above, AR binds to the consensus ARE to induce the androgens' effects. However, several non-consensus ARE have also been reported in rat genes. The rat probasin gene contains a 17 bp non-consensus ARE (ARE-2) consisting of 5'-GTAAAGTACTCCAAGAA-3' (91). This element is located within the 5'-UTR and is approximately 100 bp downstream of a consensus ARE. Even though both the non-consensus and consensus elements bind AR, neither is an effective independent enhancer when tested with a heterologous thymidine kinase promoter. However, when they are both present in the same construct, a marked response to androgen is observed (91). The androgen-regulated 20-kD protein gene, which consists of four exons that code for a major secretory protein of rat ventral prostate, contains both consensus and non-consensus ARE (92). These are present in the first intron (In-1) and when CV1 cells were cotransfected with either GR or AR, In-1 and its most active subfragment, In-1c functioned as AREs but not as GRE (92). This effect was diminished in PC-3 and HeLa cells suggesting that other transcriptional factors may confer AR specificity to the non-consensus ARE. The human glandular kallikrein-1 gene (*hKLK-2*) contains an ARE in its 5' promoter region consisting of 5'-GGAACAGCAAGTGCT-3' (93). Deletion of the promoter immediately 5' to the non-consensus ARE

resulted in a decreased induction of CAT activity (93). This suggests that the non-consensus ARE activity may be dependent on the interaction of AR with transcription factors which may bind to these upstream sequences.

In summary, it is likely that AR interaction with non-consensus ARE may depend on the interaction with other transcription factors which bind to cis-acting DNA elements in close proximity to the ARE. The existence of non-consensus ARE may allow androgen:AR complexes to expand their transactivating action. Thus, in conjunction with the classical AREs of androgen responsive genes, the non-consensus ARE may provide a degree of variability for the control of hormonal responses. Further identification and characterization of non-consensus ARE will provide additional clues as to how these elements participate in gene regulation.

7. HOW DOES A NONSPECIFIC STEROID RESPONSE ELEMENT CONFER ANDROGEN SPECIFIC ACTION?

Characterization of the consensus ARE using the AR DNA binding domain (DBD) in a DNA binding-site selection assay revealed the consensus ARE to be identical to the consensus GRE (94). This finding, taken together with the observation that GR is nearly ubiquitous in cells elicits the question as how androgen specific action is mediated by the ARE. Several observations provide clues as to how specific action can be mediated from these apparently non-specific HREs. Many androgen responsive genes have promoters which consist of several regulatory elements which suggests that an androgen specific response may be due to a combination of interacting transcription factors. This control mechanism would allow for developmental and cell specific control of androgen response as is observed with the 20 kDa protein gene (92). That the receptor itself may interact differently at the same response element is suggested by the observation that even though the DBDs of members of the GR subfamily are highly conserved, the transactivation domains are not. Thus, even though GR and AR may bind to the same DNA element, they may invoke different activity on the promoter region. This is the case for the sex-limited protein (Slp) of the mouse (95). Slp is a murine serum protein of unknown function which is synthesized primarily in the liver of mature males or T-treated females. It is encoded by a gene (C4-slp) in the S region of the major histocompatability complex class III gene complex (95). Slp's androgen dependence has been attributed to an androgen-responsive promoter in the C4-slp gene. Hemenway *et al.* identified several DNAse I hypersensitive sites which appeared in the C4-slp promoter upon androgen treatment (96). An androgen responsive region was identified within a 750 bp fragment found 2-kb upstream of the transcription initiation site in the C4-slp promoter by Loreni *et al*. (97). This androgen-responsive enhancer was identified as a 5' long terminal repeat of an ancient provirus which is most likely a retrotransposon. Adler *et al.* delineated the androgen-responsive enhancer to a 160 bp fragment (98).

Even though both AR and GR can bind the HRE element in the context of the slp promoter, GR inhibits AR induction of the slp gene (99). Interestingly, the GR can activate a heterologous promoter (thymdine kinase promoter) when the slp HRE element itself is placed upstream of it. Additionally, when the HRE element is moved as little as 10 bases away from the enhancer region, GR can activate the promoter (100). These findings strongly support that the context of the HRE is important in determining the specificity of the steroid receptor response. In light of the multiple elements found in the promoter region, such specificity is most likely mediated by protein-protein interactions with accessory transcription factors. These factors either inhibit or are not sufficient for the GR-induced activation of the enhancer while they are permissive for the AR-induced activation. Differences in transactivation domains of the steroid receptors could account for the distinct interactions which occur with the accessory transcription factors.

Tissue specific expression of hormone receptors is another mechanism by which a steroid-specific response can be generated. Strahle *et al.* introduced a progesterone-receptor expression plasmid into the rat hepatoma cell line, Fto2B-3 which contains glucocorticoid receptor but is devoid of progesterone receptor (101). They observed that the expression of the progesterone receptor in Fto2B-3 cells rendered endogenous glucocorticoid-regulated genes inducible by progestins. This experiment demonstrated that the specificity of responsiveness by a HRE to a steroid receptor in cells can be reprogrammed by the expression of a different steroid receptor which is capable of binding the HRE in question.

In summary, steroid specific expression can be mediated at both the gene and cellular levels. At the gene level, the context of the HRE may be critical for orchestrating a specific steroid response. Response elements in the proximity of the HRE may provide binding sites for accessory transcription factors. These factors are required to generate activation of the enhancer and yet may only interact with a specific hormone receptor, generating the specificity of the steroid-induced gene activation. At the cellular level, tissue specific expression of steroid receptors will limit the steroid response only to those cognate receptors.

8. HOW DOES PROSTATE CANCER ACQUIRE ANDROGEN INDEPENDENCE?

When initially diagnosed, the majority of prostate cancers respond to androgen deprivation; however, over time they lose their dependency on androgens and the tumor recurs. The molecular events responsible for development of androgen independence are currently unknown. Altered AR expression or function, loss of tumor suppressor genes, and overexpression of or altered response to growth factors may all be implicated in this phenomenon.

AR is an important mediator of early prostate cancer growth. This is underscored by the marked tumor regression associated with androgen deprivation. However, the importance of the AR during the late stages of prostate cancer is not very clear. Initial studies demonstrated that AR mRNA is present in androgen-sensitive prostate cancer cell lines but is absent or is expressed at low levels in androgen-independent lines (102). Sato *et al.* demonstrated that as prostate cancer progresses to a less differentiated state AR expression was decreased (103). Recent immunohistochemical studies have revealed that the total AR content of prostate cancer cannot be used for assessment of prognosis; rather the heterogeneity of AR expression throughout the tumor was significant as a predictor of response of tumor to hormonal therapy (104-106). Chodak *et al.* also demonstrated that AR was uniformly present in the nuclei of epithelial cells in normal prostate, whereas in prostatic adenocarcinoma only some of the epithelial nuclei stained for AR (104). Sadi and Barrack demonstrated that cancer cells in patients with advanced prostate cancer who responded poorly to therapy exhibited a significant heterogeneity in the staining intensity of the AR (105). These observations suggest that AR expression may be modified during prostate cancer progression and may somehow be associated with the development of androgen independence. One must bear in mind that these studies do not eliminate the possibility that a mutated form of the AR may be expressed that no longer reacts with the antibodies to AR.

AR mutation may confer androgen independence on prostate cancer cells. Although mutations of the AR in prostate cancer are not often identified, they have been reported in up to 25% of the patients in one study (107). A variety of unique mutations, typically occurring in the ligand-binding domain, have been found in prostate cancer (103, 108-111). The LNCaP AR has a single point mutation in codon 877 (ACT→GCT) changing Thr to Ala in the ligand binding domain (112). This mutation decreased dependency of AR activation on androgen because progestagens, estradiol and anti-androgens could compete with androgens for binding to the androgen receptor and could stimulate both cell growth and secretion of prostate specific acid phosphatase (113, 114). This mutation may have a biologic relevance *in vivo* since Gaddipati *et al.* reported that 6 of 24 prostatic tissue specimens derived from transurethral resections of patients with metastatic prostate cancer had AR mutations identical to those found in the LNCaP cell line (107). In addition to activation of mutated AR by a greater number of ligands, the AR can be mutated in such a fashion that it is constitutively active (6). *In vitro* induced deletion of the AR's ligand-binding domain resulted in an AR that in the absence of steroid possessed up to 90% of the activity a steroid-induced wildtype AR (6). Though not observed in prostate cancer, a constitutively activated AR would circumvent the need for androgen, resulting in a phenotype of androgen independence.

A variety of growth factors have been implicated in either autocrine or paracrine prostate cancer cell growth (reviewed in (115)). Culig *et al.* provided compelling evidence for the ability of various growth factors to activate androgen responsive genes via AR in prostate cancer cells (116). Insulin-like growth factor I (IGF-I), epidermal growth factor (EGF), or keratinocyte growth factor (KGF), stimulated an AR-responsive reporter gene used to cotransfect DU-145, an AR negative prostate cancer cell line, with an AR expression vector. In the absence of the AR expression vector, the reporter gene was not activated. Furthermore, exposure of LNCaP cells to IGF-1 resulted in expression of prostate-specific antigen, which is encoded by an androgen responsive gene (93, 117). These actions were blocked by casodex, a pure AR antagonist. These experiments suggest that androgen independence could arise as a result of overexpression of growth factors in the local environment of the prostate.

In summary, the molecular events which facilitate development of androgen independence are currently unknown. However, loss or mutation of androgen receptor may play a role in selection of androgen-independent tumors. Such mutations in the ligand binding domain of the AR may result in a promiscuous response to non-androgen ligands allowing prostate cancer proliferation in response to non-androgenic growth factors.

9. SUMMARY

We have reviewed several critical issues concerning the cellular and molecular biology of androgen regulation. Androgens, working via the AR, afford multiple levels of regulation. The levels of control include hormone metabolism, AR affinity for ligands, HRE, and mesenchymal-epithelial interactions. The overall effect of these phenomena may be the basis for the diverse response of tissues to androgen. Future studies on androgens and AR

should elucidate the importance of protein-protein interactions and the role of ARE in imparting control over gene expression specificity. Other areas of research including analysis of receptor phosphorylation, cell cycle biology, and gene methylation will undoubtedly contribute to our understanding of the androgens' effect on expression of diverse androgen responsive genes.

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