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REVIEWS

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# AMPLIFICATION AND CO-REGULATORS OF ANDROGEN RECEPTOR GENE IN PROSTATE CANCER

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**Prostate cancer is the second most common malignancy among males after lung cancer. The growth of prostate cancer cells depends on the presence of androgens, a group of steroid hormones that include testosterone and its more active metabolite dihydrotestosterone. Most prostate cancers are androgen-dependent and respond to the antiandrogens or androgen-deprivation therapy. However, the progression to an androgen-independent stage occurs frequently. Possible mechanisms that could be involved in the development of hormone resistant prostate cancer causes including androgen receptor (AR) mutations, AR amplification/over expression, interaction between AR and other growth factors, and enhanced signaling in a ligand-independent manner are discussed.**
***Key Words:*** **prostate cancer, androgen receptors, co-regulators.**

## ANDROGEN RECEPTOR AMPLIFICATION

The significance of androgens in the development of prostate cancer has been known for more than half century. During the last decade, a lot of efforts has been made to study the significance of the specific nuclear receptor of the hormone, androgen receptor (AR). It has been suggested that polymorphisms, especially the length of CAG repeat in exon 1 of the gene, are associated with the risk of prostate cancer. However, not all studies have confirmed the association. Most surprisingly, it has now become clear that prostate carcinomas emerging during the androgen withdrawal therapy (i. e. hormone-refractory tumors) are capable of reactivating the AR-mediated signaling despite of the low levels of androgens. In addition, it has been shown that *AR* gene itself is genetically targeted. Androgen receptor gene amplification (ARGA) has been suggested as one of the molecular mechanisms responsible for the development of hormone refractory prostate cancer. Progression of prostate cancer during endocrine therapy is a major clinical problem, the molecular mechanisms of which remain poorly understood. Amplification of the *AR* gene was recently described in recurrent prostate carcinomas from patients who had failed androgen deprivation therapy [1, 2]. According to several studies, the frequency of ARGA is stemmed to be around 30%. On the other hand, insignificant differences were found between AR expression in tumors with and without gene amplification and not all prostate tumors with ARGA showed increased levels of the AR protein. These data suggest that other mechanisms apart from AR amplification are involved in the progression of prostate cancer. An association between ARGA and response to therapy has been demonstrated. In patients who assumed combined androgen blockade after initial androgen deprivation, a major response was observed in those with ARGA than in those without ARGA [2, 3]. In the same study decreased PSA levels were found in patients with ARGA compared to patients without it [1, 2]. In contrast, in the bibliography we find evidence that between PSA expression and ARGA any correlation might exist. To establish the link between prostate specific antigen PSA and gene amplification further investigations are required. During neoplastic progression, other gene amplification such as *p53*, *myc*, *CCND1* or *ErbB2* may occur. A recent study provides evidence of an increasing *p53* expression during progression from an androgen dependent to an androgen independent prostate cancer, and positive $p53^+$ tumors were found more frequently in patients with ARGA than in patients without it. Furthermore, amplification of *myc* and *ARGA* genes was detected in 11% and 22% cases, respectively in metastases from patients with hormone-refractory prostate cancer, in comparison to locally recurrent tumors (4–23%). In summary, neoplastic progression depends on the accumulation of multiple genetic alterations some of which may occur at early stages and others — at late ones. Each molecular event can influence cell-cycle and apoptosis, and may play a role as prognostic factor in prostate cancer [4–6].

## CO-REGULATORS

There is an evidence that the AR transcriptional activity is influenced by several co-regulators. These molecules can either up-regulate (co-activators) or down-regulate (co-repressors) the AR transcriptional status, usually in a ligand-dependent manner and generally without binding to DNA. Some of them such as cyclic AMP binding protein (CBP)/p300 and steroid receptor coactivator-1 (SRC-1) can induce DNA modification recruiting the CBP/p300-associated factor, which possesses histone acetyltransferase (HAT) activity. Furthermore, the finding that hydroxyflutamide can increase AR activity when CBP is over

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*Abbreviations used:* AR – androgen receptor; ARA – androgen receptor – associated coregulator; ARGA – androgen receptor gene amplification; CBP- cyclic AMP binding protein; DHT – dihydrotestosterone; FHL2 – four and a half LIM domains 2; GF – growth factors; HAT – histone acetyltransferase; MAPK – mitogen activated protein kinase; PHD – plant homodomain; PSA – prostate specific antigen; SRC-1 – steroid receptor coactivator-1.

expressed confirms the hypothesis that co-regulators can interfere with the effects of antiandrogens by altering the AR ligand specificity and thus contributing to the progression of prostate cancer [7, 8]. Various studies reported different results regarding the co-regulators expression in prostate cancer. Scientists by using RT-PCR method found that ARA54, ARA70, and SRC-1 were expressed in both tumor and normal cultured prostate cells. ARA55 was not expressed in androgen-independent LNCaP and DU145 prostate cancer cell lines, while its very low levels were detected in PNT2 cell line [9, 10]. Additionally, other studies have demonstrated, by the method of *in situ* hydritization, higher levels of the co-regulator Ran/ARA24 in prostate tumor specimens. In contrast, other scientists did not find overexpression of ARA24 using RT-PCR in prostate cancer xenografts and cell lines. Perhaps this discrepancy could be explained by the fact that *in situ* hydritization is a poor quantitative method. Also, SRC-1 expression appeared to be reduced. This finding correspondes to the results of another group of scientists who demonstrated lower SRC-1 expression in hormone refractory LNCaP cells in comparison to hormone dependent LNCaP cells [9–11]. In contrast, another study detected elevated levels of SRC-1 protein in androgen independent prostate cancer [10, 12]. Differences in cell cultures conditions, cell density, methods of analysis and various types of cell lines may explain these divergent results. However the weight of evidence sustains the expression and the biological role of co-regulators in AR modulation and promotion to advanced prostate cancer.

### *ARA54*

Ara54 is a protein of 474 aminoacids and molecular weight of 54kDa that is shown to increase AR transcriptional activity in a DU145 cell line. ARA54 contains a conserved RING finger motif and a B-box like structure. It has been reported that the C-terminal region, without the RING finger motif, may exert inhibitory effect on AR-mediated transactivation. ARA54 may also enhance the transcriptional status of LNCaP mutant AR (ART877a) but not wild type AR or another mutant AR (Are708k). A mutant ARA54 form (mt-ARA54), with a point mutation at aminoacid 472 and incapable to bind to AR, can suppress the positive AR transactivation of endogenous or exogenous full-length f-l ARA54. Furthermore, the inhibition was higher for exogenous f-l ARA54 in DU145 cells than for endogenous in PC-3 and LNCaP cells, probably because of the intervention of other coactivators in PC-3 and NCaP cells. Moreover, mt-ARA54 disrupts the interaction between lf-ARA54 and fl-ARA54 molecules by forming dimers with fl-ARA54, suggesting that ARA54 may need to form homodimers to increase AR transcriptional status. These findings underline that dominant-negative mutant forms of ARA54 or of other co-regulators, should be considered as potential targets for prostate cancer therapy [9, 13].

### *ARA55*

ARA55 consists of 444 aminoacids and has a molecular weight of 55 kDa. There is a sequence homology between human *ARA55* gene and mouse TGF-β1 inducible gene *hic-5*. ARA55 seems to have a role in the stromal epithelial interaction involved in developing human fetal prostate. ARA 55 was found to be expressed in stromal cells with a zonal pattern, primarily in the peripheral zone surrounding the non canalized acini. Tissue distribution studies suggest that ARA55 may be differentially expressed during various stages of prostate cancer. Higher ARA55 levels were detected in tissue specimens from androgen-independent prostate cancers than in those from androgen-dependent prostate cancers [10, 14, 15]. In contrast, using RT-PCR scientists found lower ARA55 levels in the tissue samples of androgen-independent prostate cancers in comparison to untreated prostate cancers or benign prostatic hypertrophy specimens [16]. On the other hand, higher ARA55 expression levels in patients with androgen — independent prostate cancer were associated with shorter recurrence free survival and overall survival. ARA55 is able to bind to AR in a ligand-dependent manner and increase its transcriptional status. The interaction occurs via the C-terminal half of ARA55, which includes three LIM motifs. Also it has been reported that ARA55 can induce AR transactivation in response to antiandrogens like hydroxyflutamide and other non androgenic steroids including 17β-estradiol. In LNCaP cells, a dominant-negative AR associated protein (dARA55) co-regulator inhibits the AR transcriptional activity and reduces the agonistic action of antiandrogens. dARA55 also inhibits PSA expression and prostate cancer cell proliferation and therefore, should be considered as a gene therapeutic agent [14–16]. A correlation was found between ARA55 and proline-rich tyrosine kinase 2 (Pyk2). Pyk2 is a member of the focal adhesion kinase (FAK) family and may be linked to the mitogen activated protein (MAP) kinase and JNK signaling pathways. As it was demonstrated in human prostate cancer cell lines, Pyk2 can directly phosphorylate ARA 55 at tyrosine 43, resulting in its inactivation. The inactivation may occur either by impairment of the activity or by sequestration of the co-activator, and the consequence is the suppression of the AR transcriptional action. However, the phosphorylation site is not found in the AR interaction domain of ARA55 (aminoacids 251–444), suggesting that other mechanisms could be involved in the suppression of the AR transactivation [17, 18].

### *ARA70*

ARA70, found in a DU-145 prostate cancer cell line, was the first AR co-regulator identified. There is a growing evidence that this protein may be implicated in the enhancement of the AR transcriptional activity through a specific ligand binding. It is reported, that it can also increase the transcriptional activity of other steroid receptors like the glucocorticoid receptor (GR), progesterone receptor (PR) and estrogen receptor (ER). However, the levels are increased slightly (up to 2-fold) in comparison to the enhance-

ment of AR (up to 10-fold). One study showed that the consensus FXXLF motif within the ARA 70-N2 domain (aminoacids 176–401) is important for the interaction with the AR. The LXXLL motifs, essential for the function of co-regulators such as the p160 co-regulator, do not have an important role in ARA70 interaction [19–21].

Whether this molecule is involved in the progression of prostate cancer is of relevant interest. The detection of ARA70 in AR-positive LNCaP cells but not in AR-negative DU 145 cells probably indicates a modification of expression and ability to interact with AR during the progression of prostate cancer from a hormone-sensitive to a hormone-insensitive state. Recently, elevated ARA70 expression was found in high grade prostate cancer tissues as well as in hormone refractory LNCaP xenografts and prostate cancer cell lines. Moreover, higher ARA70 protein levels (91.74%) were detected in prostate cancer specimens than in benign tissue (64.64%). In addition, one study demonstrated enhanced ARA70 mRNA levels in a recurrent androgen-independent CWR22 prostate cancer xenograft, derived from an androgen-stimulated state after castration. ARA 70 has been extensively examined as a molecule that may potentiate AR transcriptional activity not only in the presence of androgens but also in the presence of antiandrogens and 17β estradiol [20]. Several studies of HeLa, PC3 and TSU-Pr1 cell lines establish the induction of AR by 17β estradiol in the presence of ARA70. However, the results of two different studies in CV-1 cells regarding ARA70 and 17β estradiol — mediated AR transactivation are contradictory. The reason of these opposing results is not yet known. Prostate cancer may continue to proliferate in response to antiandrogens (hydroxyflutamide and bicalutamide) and the role of ARA 70 is critical for this process. In LNCaP cell line, the addition of a dominant negative AR co-regulator ARA 70 (aARA70N), lacking AR interaction, blocks the ARA70-enhanced AR transcriptional activity by forming a non-functional heteromer with ARA70. The RNA-interference-mediated silencing of ARA70 gene confirms this observation [19, 20–23].

### *ARA267-a*

ARA267-a is an AR co-regulator containing the SET domain with 130 aminoacid motif named from three originally identified proteins, Su(var)3–9, Enhancer-of-zeste and Thritorax. It also contains two LXXLL motifs, three nuclear translocation signal (NLS) sequences and four plant homodomain (PHD) finger domains. Recent data show that ARA267-a can increase the AR transactivation in prostate cancer cells, probably by binding to DNA and remodeling of chromatin structure. The SET domain and the PHD fingers may play important roles in AR-mediated gene transcription. In addition, it can not be excluded that the LXXLL motifs, motifs essential for the function of the co-regulators SRC-1 and TIFII, may also be important for the ARA267-a function. Finally, according to the results of a recent study, an interaction between DR6cp (a member of the TNF receptor family that mediate cell apoptosis) and ARA267-a fragment containing four PHD and one SET conserved domains may occur, suggesting a possible cross-talk between the apoptosis signaling pathway and the androgen signaling pathway [24–26].

### *ART-27*

Art-27, a small protein of 157 aminoacids and with molecular weight of 18 kDa, is a newly identified AR N-terminal coactivator that increases receptor-dependent transcriptional activity. It seems to interact predominantly with the AR aminoacids 153–336, containing AF-1a and a part of AF-1b. Art-27 is expressed in a variety of human tissues sensitive to androgen action such as prostate, breast and skeletal muscle. According to the results of some studies, Art-27 promotes epithelial prostate cell differentiation and inhibits proliferation. In human prostate cancer cells this protein in not markedly expressed. In LNCaP transfected cell line, the reintroduction of Art-27 reduced cell proliferation and upregulated the androgen-mediated transcription of the *PSA* gene, a gene that is activated in differentiated epithelial prostate cells. The mechanism by which Art-27 is regulated is unclear. However, androgens might not directly control its expression because of the restricted cell-type distribution of Art-27. Moreover, addition of androgens in LNCaP cell line did not induce Art-27 expression. The altered Art-27 expression during prostate cancer progression should be an object of further analysis [27–29].

### *β-Catenin*

β-Catenin contains five LXXLL motifs situated in the central core region containing the armadillo repeats. This region is required for the β-catenin interaction with E-cadherin and members of the T-cell factor (TCF) and lymphoid enhancer factor (LEF) family, thus initiating the transcription of the Wnt/Wingless-responsive genes [30, 31]. The Wnt/Wingless signaling pathway apart of its role in regulating cell processes such as proliferation, polarity and migration, is also involved in oncogenesis. 80% of colon cancers present elevated β-catenin-TCF signaling. Moreover, it has been demonstrated that β-catenin mutations can occur in more than 50% of colon cancer cases and in more than 50% of hepatoblastoma cases. β-catenin mutations were detected in 5% of prostate cancer tissue samples. Four of them are located in the serine or theonine residues implicated in the degradation of β-catenin and one (the codon 32) changes aspartic acid to a tyrosine. The mutations occurred focally and therefore should be considered as a late event in prostate cancer progression. β-catenin can regulate AR function and contribute to the prostate cancer progression despite low levels of endogenous androgens, by modulating receptor-dependent signaling. In LNCaP cell line, β-catenin (β-catenin S33F) can relieve the inhibitory effects of the antiandrogen bicalutamide and increase the poor action to the androgen androstenedione on AR transactivation to a level comparable to DHT. Correlation between β-catenin and other steroid receptors

is possible: it increases the transcriptional activity of the retinoic acid receptor [31, 32].

### *FHL2*

Four and a half LIM domains 2 (FHL2), a LIM protein, is the first tissue-specific co-activator of the AR. As it was shown for prostate epithelial cell and the myocardium of the heart, FHL2 is able to enhance the AR transactivation in an agonist- and AF-2-dependent manner. The interaction between FHL2 and AR requires both the N- and C-terminus of the AR. There is also evidence of an interaction between FHL2 and β-catenin. Although no synergistic action on AR transcriptional activity was observed in prostatic cell cultures, the activation of the cyclin D1 promoter is mediated by FHL2 in a β-catenin dependent manner in liver tumors. It was shown that the Rho signaling pathway induces activation and nuclear translocation of FHL2, because AR-dependent genes activation and the the N-terminal of FHL2 are required for this process. In addition, Rho GTP-ases overexpression and FHL2 nuclear localization correlate with a lower differentiation grade of prostate tumor. Therefore, it would be interesting to examine the contribution of FHL2 to AR function and prostate cancer progression [33–36].

### *Her2/NEU*

Her2/Neu, a transmembrane glycoprotein with intrinsic tyrosine kinase activity, is a member of the epidermal growth factor receptors family (EGFR). Overexpression of Her2/Neu was found in 30% of breast and ovarian cancers. Contrasting results of various studies regarding the Her2/Neu gene product expression are reported, probably due to differences in the reagents, lack of standardized techniques and different scoring methodologies. Moreover, by several researches a varying degree of Her2/Neu gene amplification was shown. However, according to the recent reports, Her2/Neu is implicated in the enhancement of AR transactivation and progression to an androgen-resistant stage of prostate cancer [37, 38].

ErbB2 is able to signal in the absence of ligand by dimerization leading to autophosphorylation and initiation of specific signal transduction cascades. There are two known transduction pathways by which Her2/Neu may lead to androgen-independent prostate cancer: the PI3K/Akt and the MAPK pathway. The Akt activation was examined in LNCaP co-trasfected cell line. It was found that Akt can be activated by Her2/Neu in the absence of androgens and that Akt can induce AR transactivation promoting the development of an androgen-independent growth of prostate cancer cells [39, 40]. In fact, growth inhibitory effects were observed when the Akt inhibitor LY294002 was added. According to the above-mentioned studies, the amino acids at sites 213 and 791 of the AR are phosphorylated by Akt. In addition, Akt may increase the PSA transcription by activating the PSA gene promoter. On the other hand, enhancement of the PSA levels and AR activity induced by Her2/Neu via the MAPK pathway were detected in LNCaP and DU145 prostate cancer cell lines. It is possible that the phosphorylation site(s) of the AR are located in the hormone-independent N-terminal region. Since there is evidence that Her2/Neu may be associated with an androgen-independent prostate cancer, numerous therapeutical molecules targeting this tyrosine kinase are in trial [39–44]. Trastuzumab (Herceptin), a monoclonal antibody directed against the extracellular domain of the Her2/Neu protein, presents antitumor activity in androgen-independent prostate cancer xenografts. Trastuzumab in association with paclitaxel, a chemotherapeutic agent, had also high antitumor properties against androgen-independent prostate cancer. Other monoclonal antibodies (MDX-H210 with GM-CSF) have been tested also with positive antiproliferative properties in DU145 and PC-3 prostate cancer cell lines since Her2/Neu protein expression was downregulated. Finally, an ansamycin antibiotic, the 17-Allylamino-17-demethoxygeldanamycin (17-AAG), can cause degradation of Her2/Neu, Akt and AR. Inhibiting action of 17-AAG was observed in prostate cancer xenografts.

### *IL-6*

IL-6, a cytokine with pleiotropic functions, plays an important role in the physiopathology of prostate cancer. Elevated IL-6 levels in the sera of patients with prostate cancer are associated with high mortality, poor prognosis and AR transactivation. IL-6 can modulate the growth of malignant cells through at least three distinct signaling pathways including JAK/STAT3, MAPK and PI3K/Akt [39, 40]. However, until now the data regarding the effects of IL-6 on prostate cancer remain controversial, probably because of the coexistence of these multiple pathways with either positive or negative influence on each other. In LNCaP cells, in the absence of androgens, IL-6 can increase *AR* gene expression and activate the AR. In addition, the application of several MAPK inhibitors blocked the IL-6- mediated induction of the androgen-responsive promoter, demonstrating that IL-6 activity depends on MAPK pathway. Many other studies report growth stimulating effects of IL-6 [40, 41, 44]. In contrast, some investigators found that IL-6 inhibits the access of the coregulator p300 to the complex p160-PSA promoter with consequent inhibition of histone acetylation [45, 46]. They also found that IL-6 inhibited the PSA gene expression, at least in part, through the STAT3 pathway without MAPK and PI3K/Akt involvement [46]. The reason for these opposite results is unknown. One study demonstrated that in LNCaP cells, IL-6 can induce G1 growth arrest reflected in decreasing levels of cyclin-dependent kinase-2 (CDK2), -4 (CDK4), and -6 (CDK6). In addition, the LNCaP cells underwent neuroendocrine differentiation. Neuroendocrine-like differentiation as a result of treatment with IL-6 was also observed by another group of scientists in an LNCaP cell line. Moreover, the scientists found that androgens-ARs can block neuroendocrine differentiation by inhibiting the IL-6 mediated PI3K/Akt pathway [39–41, 45, 46].

## CONCLUSION

Growth of the prostate malignant cells highly depends on the androgens presence. ARGA represents one of the molecular mechanisms involved in the development of hormone refractory prostate cancer. AR trancriptional activity is influenced by a number of co-regulators. Co-activators and co-repressors impair the AR transcriptional status, which in return affect the whole process of prostate carcinogenesis. ARA54, ARA55, ARA70, ARA 267-a, ART-27 as well as β-catenin, FHL-2, Her2/Neu and IL-6, represent the most important and well-studied co-regulator proteins.

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