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ARTICLE

# Androgen Receptor Expression in Relation to Apoptosis and the Expression of Cell Cycle Related Proteins in Prostate Cancer

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**The expression of several genes involved in the regulation of cell cycle and apoptosis may be regulated via the androgen receptor (AR) in the prostate. AR may have a role in the prognosis of prostatic carcinoma. The aim was to examine AR expression status and its relationship with markers of proliferation, apoptosis and cell cycle control in prostate cancer. Expression of AR, bcl-2, bax, Ki-67 and p53 was examined in paraffin-embedded tissues from 50 cases of prostate carcinoma by immunohistochemistry and evaluated using an index of staining. Detection of apoptotic cells was performed by TUNEL method. Correlation between AR expression and apoptosis, proliferation index, bcl-2, bax and p53 and also clinicopathological parameters including stage, pathological grade and Gleason score were determined. AR expression was observed in all cases with mean expression of 81%±15 and mean staining index of 141±65. No correlation was found between AR expression and apoptosis detected in patients. The mean AR staining index was 170±72 in bcl-2 positive tumors versus 120±53 in bcl-2 negative tumors showing a significant association between AR and bcl-2 expression (p=0.015). AR expression also showed a significant association with bcl-2/bax ratio (r=0.321, p=0.023) and Ki-67 proliferation staining index (r=0.396, p=0.004). Although a significant correlation between Ki-67 and p53 with differentiation status of the tumors was observed (p<0.004) no correlation was found with AR. AR expression showed no prognostic value regarding its correlation with stage and differentiation status of the prostate carcinoma. However, its significant correlation with Ki-67 and bcl-2 that are markers of cell survival suggest its contribution to tumor cell progression.** (Pathology Oncology Research Vol 10, No 1, 37–41)

*Keywords:* prostate carcinoma, androgen receptor, apoptosis, p53, bcl-2, bax, Ki-67

## *Introduction*

The prostate gland requires stimulation by androgenic steroids for its embryological and post pubertal development for its growth, vitality and function.[1] Androgen action requires intracellular androgen receptor (AR) protein that is a typical member of the steroid receptor family.[2] AR is located on the nucleus of the epithelial cells as well as some stromal cells.[3] Steroid binding to AR could stimulate proliferation and differentiation of epithelial cells and inhibition of prostate cell apoptosis.[4] Studies of rodent models have shown that decrease in circulating steroids will rapidly induce apoptosis of the epithelial cells of prostate leading to extensive glandular regression.[5] It is assumed that androgen might regulate the synthesis of key elements involved in the signaling pathways of proliferation distinctly from apoptosis.[6] Apoptotic cell death in prostate has been shown to accompanied or preceded by an elevated expression of the c-fos proto-oncogene that can initiate alteration in the synthesis of p53 and other apoptosis-regulating gene products such as bcl-2 family members.[7]

In malignant tumors of the prostate, elimination of tumor cells following withdrawal of androgens is the base of the hormone ablation therapy.[8] However, this only causes a temporary regression of prostate tumors and some tumor cells become androgen independent and resist to therapy.[9] Changes in the androgen receptor gene expression have been observed to be involved in the

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development of androgen–independent growth in prostate cancer,[10] therefore, evaluation of AR expression may be useful for estimating the prognosis of the patients.[11] AR has been reported to be present on most of prostate tumor cells by immunohistochemistry, but the percent of positive nuclei and the intensity is varied between different patients.[12-13] In the present study the expression of AR and its relationship with apoptosis, apoptosis markers (bcl-2, bax), proliferation marker (Ki-67) and cell cycle regulator molecule, p53 as well as patient's characteristics were studied by evalnating a staining index in which both percentage and intensity of staining is included. To the best of our knowledge these markers have not been considered for study together in prostate cancer before.

## *Materials and Methods*

### *Samples*

179 patients proven to have prostate adenocarcinoma were selected from the pathology archives of Shiraz University affiliated hospitals. Among them 50 untreated patients with adequate clinical and paraclinical information were chosen. The best paraffin-embedded block of neoplastic tissue was processed for further studies. The neoplastic tissues were either from tumor biopsies, transurethral resection of prostate or prostectomy speciemens. 5- m sections were cut for histological evaluation by routine hematoxylin and eosin staining as well as immunohistochemical staining. Samples were graded according to Gleason score. The average patient age was 72.8±7.8 years (range 60-90 years). Information on patient stage based on TNM classification was obtained from the hospital clinical records.

### *Immunohistochemical analysis*

Strepavidin-biotin immunoperoxidase staining was performed on all cases. The mouse monoclonal anti – androgen receptor was purchased from Dako, Denmark. Monoclonal anti-p53 antibody DO1 was kindly provided by Dr Nouri AME, The Royal London Hospital, London. The mouse monoclonal antibody bcl-2 (1:200, Dako, Denmark) was used to detect bcl-2 expression. The polyclonal antibody to Ki-67 (Dako) was used to identify proliferative activity. Polyclonal rabbit anti-human bax was purchased from Dako. Bax expression was performed using DAKO-Catalyzed signal amplification (CSA) system. Briefly, after tissue sections were deparaffinized and rehydrated, they were incubated with 3% $H_2O_2$ to inactivate endogenous peroxidase activity. Slides were heated in 10 mM citrate buffer to enhance antigen retrieval. Following a 20 minute blocking step with 10% normal horse serum diluted in phosphate-buffered saline (PBS), the primary antibodies were applied and incubated for overnight. After treating with biotinylated anti mouse IgG and avidin-peroxidase for 30 minutes each, diaminobenzidine 1mg/ml in PBS containing 0.03% hydrogen peroxide was applied as the chromogen. Sections were counterstained with hematoxylin for 15 seconds. For each antibody negative controls studies were performed in which PBS was used instead of primary antibody. Paraffin sections from human tonsils and breast cancer and colon adenocarcinoma with known immunoreactivity to bcl-2, p53 and Ki-67 antigens, were used as positive control respectively. All immunostained slides were analyzed and scored by two researchers in a blinded fashion without knowledge of clinicopathological data (grade and stage). To determine the expression of markers at least 1000-tumor cells were assessed and the proportion of cells showing reactivity was determined. Immunostaining intensity was also rated as follows: 0 none, 0.5 very week, 1 weak, 2 moderate and 3 strong. An index of staining was determined by multiplying the percentage and intensity of positive tumor cells.

### *Apoptosis detection*

Apoptotic carcinoma cells were identified using the Cell Death Kit (Boehringer Mannheim GmbH, Germany) as recommended by the manufacturer. Briefly, sections were deparaffinized and rehydrated, and the endogenous peroxidase activity was blocked. The sections were incubated in proteinase K then incubated with 50 l of TUNEL reaction mixture. Following rinsing in PBS, slides were incubated with 50 l converter-peroxidase solution. The reaction was visualized with diamineobenzidine/$H_2O_2$. Slides were subsequently washed and counterstained. Negative control sections included the above process except the enzyme solution and positive control were prepared by treating sections with 1 g/ml DNase 10 minutes before the above protocol. Apoptotic cells were identified using light microscope, and a total of 1000 carcinoma cells were evaluated, whereby the apoptotic index was determined

### *Statistics*

SPSS version 10 and Spearman correlation coefficient was used for Statistical analysis.

## *Results*

Fifty formalin fixed, paraffin-embedded specimens of prostatic cancer were analyzed for the pattern of expression of AR and a range of markers including bcl-2, bax, p53, Ki-67 as well as apoptosis. The immunohistochemistry results were evaluated in terms of the percentage of immunopositive tumor cells and relative immunointensity. An immunostaining index was calculated for each marker. Of 50 cases, 32% were well differentiated (Gleason score

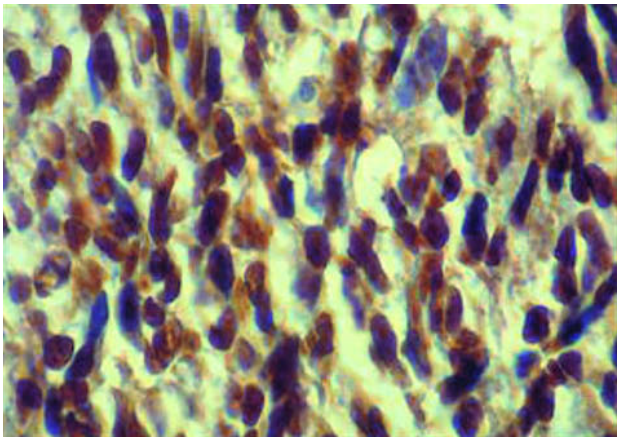

***Figure 1.*** *Nuclear staining of androgen receptor (AR) in prostate adenocarcinoma. x400.*

2-4), 54% moderately (5-7) and 14% were poorly differentiated (8-9). Staging was available on 62% of the cases in that 29% were stage B, 38% stage C and 33% were clinically in stage D.

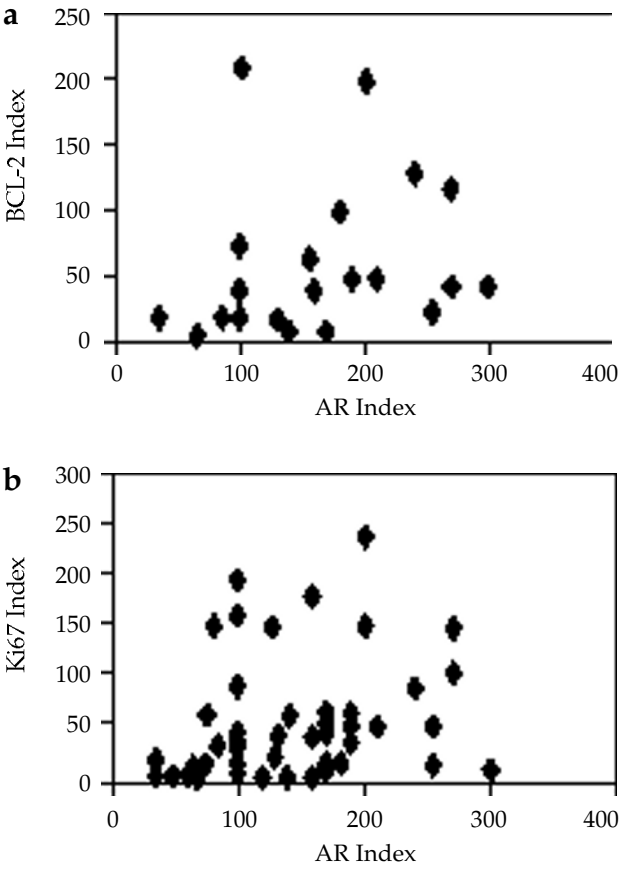


***Figure 2.*** *Relationship between AR expression index and* ***(a)*** *bcl-2 and* ***(b)*** *Ki-67 staining indices in patients with prostate carcinoma (p=0.015, p=0.004, respectively).*

*Androgen receptor expression*

100% of cases were positive for AR (35-100%) with mean expression of 81%±15 (*Figure 1*). Staining indices were between 50–300 with an average of 141±65. AR expression showed no correlation with patients' data including pathological grade, Gleason score and stage.

*Apoptosis and AR expression*

Apoptosis was detected in 12% of prostate carcinoma samples. The mean staining index was 31±28. Study of the relationship between AR expression and apoptosis showed no significant correlation.

*AR and bcl-2 and bax expression*

Cytoplasmic staining of bcl-2 was observed in 42% of cases. The bcl-2 staining index was 61±59. The mean AR staining index was 170±72 in bcl-2 positive tumors versus 120±53 in bcl-2 negative tumors showing a statistically significant association between AR and bcl-2 expression (r=0.343, p=0.015) (*Figure 2a*).

96% (48) of cases showed immunoreactivity for bax. The average value of bax expression was 102±68. No association between AR and bax expression was observed. Study of the relationship between bcl-2/bax ratio and AR expression showed a significant correlation (r=0.321, p=0.023) indicating the greater expression of AR in tumors with higher bcl-2 and bcl-2/bax ratio.

*Proliferation index and AR expression*

Ki-67 expression was observed in 48 cases with a mean staining index of 54±59. A significant correlation between Ki-67 expression Gleason score and pathologic grade was observed (r=0.437, p=0.002 and r=0.451, p=0.001, respectively) showing an increase in the expression of Ki-67 in less differentiated tumor cells. AR expression showed a good correlation with proliferation index (r=0.396, p=0.004) (*Figure 2b*) as the mean AR staining index was higher in tumors with higher Ki-67 staining index (e.g. 169±60 in tumors with Ki-67 staining index = 50 and 124±62 in tumors with Ki-67 index < 50).

The ratio of apoptotic cells to Ki-67 positive cells was determined. Although, AR was expressed more in cases with higher apoptosis/Ki-67 indices ratio (r=0.441) the difference was not significant.

*P53 and AR expression*

P53 was over expressed in 96% of cases ranging from 26 to 100%. Nuclear staining index of p53 varied 13 to 300 with an average of 102±68. AR expression was more

detected in cases with higher p53 tumor stained cells. As, study of AR expression in cases with p53 staining index more than 100 showed more AR expression than other cases (157±70 versus 133±61).

### *Discussion*

Androgen receptor, a transcription factor that mediates the action of androgens in target tissues seems to be crucial for prostate cell survival.[14] In the present study the role of AR expression in relation to clinicopathological characteristics of patients with prostate carcinoma as well as its relationship with apoptosis and a combination of markers involved in apoptosis and cell cycle control were examined. Immunohistochemical study of AR showed a positive nuclear staining in all cases with mean expression of approximately 81%±15 and an average of 141±65 staining index. In a previous study 85% of the prostatic carcinomas showed high levels of expression, defined as having AR present in more than 50% of the cells.[15] Nuclear staining of AR with a mean percent of 53.6% immunoreactivity was also observed in all cases studied by Sweat et al.[16] In another study determination of the labeling index of the AR stain values in prostate cancer was 57.8% in 86 patients.[17] Over 80% of the tumor cells in 13 of 17 examined tumors were positive for AR in another study.[18] These results show that AR expression could be detected in most prostate cancer with various degrees of staining.

Attempt has been made to find the prognostic significance of AR expression in prostate cancer. Higher AR content in cases with a low Gleason score than those with a high Gleason score has been reported previously.[19] We didn't find any correlation between AR expression and the pathological grade, Gleason score or stage of carcinoma. This may be in part due to the method of AR expression evaluation or other factors including genetic background or ethnicity involved in prostate cancer tumorigenecity.

In our study a significant association between AR and bcl-2 expression as well as bcl-2/bax ratio was observed that could be an indicator of AR role in regulating bcl-2. It has been demonstrated that overexpression of bcl-2 protects prostate cancer cells from apoptosis.[20] Bcl-2 gene family is implicated in the development of hormone refractory prostate cancer and resistance to anti-cancer therapy.[21] In this regard a weak association between AR and bcl-2 and a significant relationship between bax expression and the AR-positive tumors in a series of patients with early hormone refractory prostate cancer has been reported.[22] In another study no association was found between the androgen receptor protein expression in bone metastases of patients with androgen-independent prostate cancer and p53 and bcl-2.[23]

No correlation between apoptosis and AR expression was observed in our study. Apoptosis was neither correlated with bcl-2 expression indicating that bcl-2 or AR is not critical factors that determine the degree of apoptosis. Lack of relationship between bcl-2 expression and apoptosis has been previously reported. [24]

Analysis of AR expression showed a significant correlation with Ki-67 proliferation index showing more expression of AR in more proliferating cells. In previous studies the relationship between AR and Ki-67 and p53 was mostly investigated after androgen deprivation therapy. Both correlations of AR gene amplifications with the proliferation rate (Ki-67 index)[25] and association between AR expression with high Ki-67 index have been reported.[26]

Tumor cell survival may be prompted by loss or change in the function of p53.[27] Several studies have reported a high incidence of p53 gene modulation in prostate cancer.[28-29] Moreover, an association between accumulation of p53 protein and androgen–independent growth of prostate cancer has been reported.[30] The significant correlation between p53 and differentiation status of carcinoma observed in our study indicates that p53 alteration is an important event in the prognosis of prostate cancer. Analysis of AR expression in our samples revealed a stronger expression in tumors with higher p53 content. Expression of the p53-mutated form that could prevent cell cycle arrest and apoptosis[31] is indicative of the presence of multiple anti-apoptotic mechanisms in prostate cancer cells. In this regard, it has been suggested that changes in bcl-2, p53, and androgen receptor may influence the threshold for apoptosis particularly in advanced prostate cancer.[32]

In conclusion, we attempted to correlate the staining index of AR and other molecules in prostate carcinoma. The association between AR expression and over expression of bcl-2 and Ki-67 that – are markers of cell survival – suggest a role for AR in proliferation and apoptotic mechanisms and that AR expression may contribute to tumor cell progression.

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